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ERRATA

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Page 17, second line above table XII, for "as of" read of this.
Page 25, citation no. 22, for "Planzen gegenüberdem" read Pflanzen gegenüber dem.
Page 25, citation no. 27, for "Planzewelt" read Pflanzenwelt.
Page 46, citation no. 1, for "pur" read sur.
Page 122, citation no. 22, for "Geschwindigheit" read Geschwindigkeit.
Page 131, citation no. 12, for "Velkens van" read Welkens von.
Page 224, first line, for "LUNDERARDH" read LUNDEGARDH.
Page 463, line 1, for "and" read und.
Page 445, line 14, citation no. 14, for "van" read von.
Page 464, line 5 from bottom, for "dien" read die.
Page 563, line 7 from bottom, for "determination" read determinant.
Page 585, line 22, for "Biogology" read Biology.
Page 760, line 11, for "3" read 1.

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PLANT PHYSIOLOGY

JANUARY, 1935

TOXICITY OF ALUMINUM ON SEEDLINGS AND ACTION OF CERTAIN IONS IN THE ELIMINATION OF THE TOXIC EFFECTS[1]

WALTER S. EISENMENGER

(WITH FOUR FIGURES)

Introduction

The physiological action of aluminum ions in a nutrient medium has been studied in humid areas for approximately 25 years. The relative abundance in the earth of this element would suggest frequent solution of portions, but the solution tension of aluminum compounds in nature prevents appreciable quantities from reaching the ionic stage. It has been found, however, that aluminum occurs in nearly all seed plants, which would indicate that the hydrogen ion concentration in the immediate area of roots is not the same as its concentration in the soil mass.

The toxicity of aluminum is not so easily determined as is the toxicity of the salts of the alkali and alkali earth elements. This is due to the fact that the salts of aluminum which are appreciably soluble are those of the strong acids, and these salts in turn are strongly hydrolyzed in aqueous solution, resulting in secondary formation of an acid. This in turn gives rise to hydrogen ions. The trials made may result in part from the measurement of the sum total of hydrogen and aluminum ion toxicity. This may occur in water or in soil media.

McLean and Gilbert (16) found that the organic compound, aluminum citrate, would in part pass through a pyroxylin membrane, and that the non-diffusible colloid portion could induce toxicity.

In general the object of these experiments was to attempt to ascertain the action of aluminum on plants. It was hoped to determine: (1) the toxicity of aluminum salts of organic acids (these salts do not precipitate aluminum hydroxide when the alkalinity is raised); (2) the counter-active

[1] Contribution of the Massachusetts Agricultural Experiment Station no. 175.

effects of aluminum toxicity by the presence of a certain anion $(OH)^-$, and cations Ca^{++} and K^+.

Review of literature

The trials to determine the action of aluminum on plants in nutrient solutions and in soil have been numerous. The harmful effects of the aluminum ion in water are not difficult to demonstrate, but when attempts are made with a three phase and heterogeneous medium such as soil, where the varied hydrogen ion concentration, adsorptive properties, varied quantities if water, and diverse activity of microorganisms enter the picture, the matter is largely one of circumstantial evidence with respect to evaluating the deleterious effects of aluminum.

The reaction of plants to aluminum is in a measure dependent upon their degree of adaptation to their habitat. Thus plants which are closely related to those of the prehistoric past subsist on less potassium, calcium, and magnesium and have in their ash abundant quantities of aluminum, iron, and silica. STOKLASA (**27**) has found that xerophytes and mesophytes are sensitive to aluminum while hydrophytes are to a greater degree resistant to the element. One of the lycopods contains slightly more than 35 per cent. of aluminum oxide in its ash, while a plant like the common pea may contain but 20 p.p.m. in the ash (**2**). Mesophytes that by chance grow in a medium of moist or marshy ground contain more aluminum than those adapted to cultivated soil.

The harmful effects of aluminum as suggested by STOKLASA (**29**) are contractions of the protoplasm, and stronger solutions may soften or even dissolve this substance. He found that when strong harmful concentrations were used there was less aluminum absorbed by the plant than when more dilute solutions were used, and from this concludes that the process is not one of simple absorption but one of alteration of permeability and disturbance of the cell colloids. This eventually leads to outward passage of calcium, potassium, and magnesium. The aluminum in turn prevents entrance of excess iron into the tissues. SKEEN (**25**) has found that calcium ions have an antagonistic effect upon the aluminum which causes the toxicity. Also MCLEAN and GILBERT (**15**) have demonstrated that aluminum poisoning was accompanied by reduced absorption of dyes, of nitrates, and of water. The aluminum was found for the most part in the cortex and concentrated in the nucleus of the protoplasm.

RUPRECHT (**23**) states that the first layer or two of cells in the growing part seem to be principally affected. The harm was probably due finally to lack of nourishment rather than to poisoning itself. SZÜCS (**31**) concludes that aluminum, like yttrium and lanthanum, has the capacity to retard plasmolysis instead of increasing permeability as was formerly thought. This

effect is to retard the relative capacity to take up other nutrients. It solidifies protoplasm. DENISON (**7**) found that aluminum salts stimulate ammonifying organisms but act adversely upon nitrifying bacteria. MCGEORGE (**13**) finds that sugar cane grown in acid soils containing soluble iron and aluminum is characterized by abnormal accumulations of these elements in the nuclei surrounding the xylem cells at the nodal point of the stalk. MAGISTAD (**17**) states that water cultures of aluminum were found to prevent the formation of lateral rootlets on barley, but not on rye.

INCREASED GROWTH RESPONSE DUE TO ALUMINUM.—ROTHERT (**22**), one of the first investigators of aluminum toxicity, reported that definite limited amounts of aluminum salts may stimulate plant development. MCLEAN and GILBERT (**16**) found that concentrations of aluminum in solutions of low concentration, such as 3–13 p.p.m., stimulate growth. SOMMER (**26**), by careful purification of salts, found that millet showed a markedly increased growth rate due to the presence of aluminum in the solution. The effect was especially marked with respect to the quality of the seeds. MAZÉ (**18**), also by using extreme care in purification of salts, found aluminum essential for adequate development of corn. STOKLASA (**28**), by using a low concentration (0.0002 atomic weight of aluminum per liter) obtained increased growth of plants as compared with those solutions containing no aluminum.

In the plant world there is obviously an extreme variance of tolerance and requirement as concerns elements; and a generalization or incompatible comparison is always misleading. The indirect effects of aluminum may not be due to the aluminum but to the acidity produced by the salt. This is especially true for the aluminum salts of strong acids.

COVILLE (**6**), in order to attain a high acidity for acid loving plants, *Rhododendron, Vaccinium, Franklina,* and *Hydrangea,* added aluminum sulphate and obtained remarkable results in soils that had become too alkaline.

CONDITIONS CONDUCIVE TO ACTIVE ALUMINUM.—Active aluminum is found more abundantly (1) in acid soils, (2) in soils low in organic matter, (3) in humid areas when the seasonal water supplies are low, and (4) in arid regions when the alkalinity becomes high. STOKLASA (**30**) states, "the richer the soil in decomposed organic matter the stronger the combination of aluminum sulphate and aluminum chloride which can be used without injury to plant life." GILBERT and PEMBER (**9**) found the dry weight yields of barley plants grown in samples of acid soils from several soil types, widely separated geographically, very closely correlated with active aluminum. This corresponds with the findings of PIERRE, POHLMAN, GORDON, and MCILVAINE (**21**). TURNER (**34**) found that aluminum is invariably actively present only in soils with pH values less than 5.1. The amount of replaceable aluminum, however, is not regular with respect to hydrogen ion con-

centration. MIYAKE (**20**) reports that the toxicity of aluminum chloride for rice seems to be approximately equal to that of hydrochloric acid of the same normality, but that the concentration of hydrogen ions found formed by the hydrolysis of aluminum chloride is less than that formed by dissociation of hydrochloric acid of the same normality.

It is stated by MAGISTAD (**17**) that data relating to the solubility of aluminum in water and soil solutions show that at pH 5 there are 1–2 p.p.m. of aluminum oxide equivalent in solution. As the acidity decreases to the neutral point the solubility decreases almost to zero. When the acidity becomes greater than pH 5 the solubility increases until pH 4.5 is reached, at which point the solubility increases rapidly. Strongly alkaline soils were found to contain much aluminum. One, at pH 9.01, contained 31 p.p.m. These data are analogous to those of LIGON and PIERRE (**11**).

MCGEORGE (**13**) states that acidity *per se* or hydrogen ion concentration of the intensity present in our most acid soils has absolutely no influence upon growth of sugar cane. It is, he states, the aluminum salts which are present in such soil types that retard plant growth. MCGEORGE, BREAZEALE, and BURGESS (**14**), in their work on electrometric titration measurements of the solubility of aluminum compounds at different hydrogen ion concentrations, conclude that from pH 5 to 7.5 aluminum is almost completely insoluble, while at pH 8 the aluminate forms. Black alkali soils contain appreciable amounts of water soluble aluminates.

TOLERANCE OF DIFFERENT PLANTS.—MCLEAN and GILBERT (**15**) found considerable variance in plant tolerance. Most sensitive are lettuce, beets, timothy, and barley; medium sensitive are sorghum, cabbage, oats, and rye; resistant are corn, turnips, and red top (the last is especially resistant). SKEEN (**25**) found the white lupine to be about three times as resistant to aluminum as is the kidney bean.

REMEDIAL MEASURES FOR ALUMINUM TOXICITY.—The suggestions that have been made for alleviating the toxicity of aluminum have been the application of neutralizing agents and buffers. Also the calcium ion has been demonstrated to overcome it (**25**). It has been found that lime, phosphate, and organic matter remedy the bad effects of aluminum. In reclamation of alkaline lands summer leaching has been suggested for elimination of soluble aluminum compounds.

Other workers have made valuable suggestions as to active aluminum occurrence and agencies for its elimination (**5, 10, 19, 7, 20, 3, 4, 1, 24**).

OPPONENTS OF THEORY OF ALUMINUM TOXICITY IN SOILS.—Owing to the apparent action of aluminum in acid solutions and soils, the question has been raised as to which ion, the H^+ or Al^{+++}, is the one that is destructive to plant tissue. Thus after years of experimentation, adversaries of the aluminum toxicity theory maintain that inaccurate observations account for

the appraisal of this element in toxicity. STOKLASA (**30**) says: "The statement that aluminum compounds in the soil have an injurious effect on plant growth is based on no exact experimental foundation." LINE (**12**) states regarding the toxic aluminum theory of soils that "old evidence and fresh experimental work seem to show that this theory is no longer tenable," that most of the aluminum in the water is in the form of colloidal hydroxide.

Methods

Aluminum citrate and aluminum tartrate were the salts used. Seedling stages of the soy bean (variety Manchu), corn (variety Sweepstakes), and Japanese buckwheat were the plants employed in the growth trials.

The seeds were germinated in a dish in the dark, and when the sprouts were about 10 mm. long they were transferred to the growth medium. The sprouts of the buckwheat were about 8 mm. long. The roots dipped into the solution.

CRITERIA OF GROWTH.—Three criteria of growth were used: (1) root elongation, (2) length of top, and (3) total dry weight. There is greater simplicity with respect to behavior in the seedling stage than in the later periods of growth. In the early stages of growth the plant is well supplied by ions, and the organic matter of the seed is sufficient to afford protection from starvation of plants grown in distilled water. The protoplasm seems to be reactive to the toxic constituents of the solution.

The investigation involved the toxicity of aluminum citrate for corn; aluminum citrate and tartrate for soy beans; and aluminum tartrate for buckwheat. The concentration of each salt ranged from 0.00012 to 0.006 M, or 2, 5, 15, 30, 50, 70, 85, 95, 98, and 100 per cent. of 0.006 M. Tests were also made to show the effects upon growth of mixtures of the aluminum salt and calcium nitrate, calcium hydroxide (freshly prepared), and potassium hydroxide. In the case of the mixtures eleven sets of percentage molecular proportions were used: 0+100, 2+98, 5+95, 15+85, 30+70, 50+50, 70+30, 85+15, 95+5, 98+2, and 100+0.

The methods relating to roots were essentially the same as those described by TRELEASE and TRELEASE (**32**) and by EISENMENGER (**8**). For each culture two pyrex beakers (tall form without lip) were used. The smaller beaker was of 300-cc. capacity, the larger of 600-cc. capacity. Over the top of the small beaker was stretched a piece of paraffined mosquito netting which was secured below the rim by a ligature of paraffined thread. The smaller beaker was placed inside the larger one, and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the seedlings from the germinating dish were of the required length they were placed on the mosquito netting so that the roots dipped

into the culture solution. Duplicate cultures of 25 plants were used for each experimental solution, a total of 50 plants for each concentration. The cultures were placed in a dark room and the seedlings were allowed to grow until the primary roots of the control culture had acquired an average length of about 95 mm., or until these roots had elongated 85 and 87 mm. (95–10 or 95–8).

The length of the primary root, the maximum length of top, and the total dry weight of each individual plant were then recorded and the average computed. From the average of root length was deducted the average length of the roots at the time the seedlings were taken from the germinating dish. The difference constituted the average root elongation value for the culture. The growth data here presented are relative values. Each relative growth value was obtained by dividing the average elongation of root, top, and total dry weight of a given culture by the average elongation of root, top, and total dry weight of the control culture and multiplying this quotient by 100. With each group were four beakers containing 25 plants each in a complete nutrient medium. These 200 plants were designated as controls. For corn the control was Knop's solution. For soy beans and buckwheat the control contained: $CaHPO_4$, 0.0011 M; $MgSO_4$, 0.0057 M; $CaSO_4$, 0.0013 M; KCl, 0.0037 M; and KNO_3, 0.007 M. There was little difference in the controls with respect to reactivity toward plants, but for soy beans the latter was slightly more favorable than was Knop's.

The temperature during the growth period of the seedlings varied from 21° to 22° C. The time required for the roots of the control solution to acquire an additional length of 85 or 87 mm. varied from 95 hours for buckwheat to 116 hours for corn. When a series of single salt solutions was used, two additional cultures of 25 plants each grown in distilled water were used. The distilled water was obtained from a Barnstead still.

Discussion

Corn

Roots.—As previously stated, corn has been regarded as one of the plants more resistant to aluminum toxicity. In the present experiment, however, 15 per cent. of 0.006 M aluminum citrate exerted a decidedly retarding effect on the growth of corn roots. This amount is approximately equal to 22 p.p.m. of aluminum (fig. 1 *B*). Amounts higher than this exerted only a slightly increased toxicity on roots compared with that at 15 per cent. of 0.006 M. The plants thus affected showed lesions of the primary root principally. The end of this root eventually assumed the nature of an open wound. The lateral roots showed no such effects except those branching from near the injury. (See table I for comparative data.)

TABLE I

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE

Roots	Tops	Dry weight	Percentage 0.006 M aluminum citrate	Reaction
%	%	%	%	*pH*
66.3	53.8	68.4	0	5.82
83.5	62.1	88.0	2	4.75
48.6	51.1	76.6	5	4.50
24.8	50.9	67.7	15	4.50
18.7	32.5	52.1	30	4.50
12.6	32.2	47.1	50	4.05
16.8	29.4	47.5	70	3.64
11.4	29.4	39.0	85	3.55
11.9	22.4	39.5	95	3.55
13.3	24.9	42.5	98	3.50
7.2	13.4	34.9	100	3.43

Roots are by far the most injured by the toxic solution; their variation due to variable concentration causes more marked breaks in the growth curve than is the case with curves of the relative length of tops and curves of the total dry weight. Thus the relative length of tops and relative total dry weight (fig. 1 *B, C*) indicate that the greatest deviation occurs between concentrations of 15 and 30 per cent. of 0.006 M, while the greatest deviation in relative root length occurs between 5 and 15 per cent. of 0.006 M.

TOPS AND TOTAL DRY WEIGHT.—The relative diminution due to aluminum citrate is greater for the tops than for total dry weight. In view of the fact that the diminution of relative length of roots is most pronounced,

TABLE II

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF CALCIUM HYDROXIDE

Roots	Tops	Dry weight	Percentage 0.006 M calcium hydroxide	Reaction
%	%	%	%	*pH*
66.4	53.9	68.8	0	6.61
93.1	55.2	75.2	2	7.85
117.1	68.4	87.5	5	7.85
121.6	74.3	97.9	15	9.0
111.7	80.2	82.6	30	9.6
80.5	63.6	88.0	50	9.6
57.7	53.9	79.5	70	9.6
32.0	28.9	62.3	85	9.6
25.0	30.6	71.1	95	9.6
26.1	37.6	69.5	98	Λ*
26.9	44.9	71.2	100	Λ*

* Λ indicates values greater than preceding figure.

TABLE III

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + CALCIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M CALCIUM HYDROXIDE	REACTION
%	%	%	%	%	pH
27.0	44.9	71.2	0	100	V*
23.0	50.0	77.0	2	98	9.0
16.8	46.6	82.0	5	95	9.0
41.7	66.5	113.4	15	85	8.8
84.5	69.2	100.7	30	70	8.37
126.7	78.3	110.6	50	50	5.60
94.5	57.2	78.9	70	30	4.18
42.2	36.1	62.5	85	15	3.70
14.0	20.6	49.1	95	5	3.40
12.4	11.3	42.5	98	2	3.40
7.2	13.4	34.6	100	0	3.43

* V indicates values above pH 9.0.

it is probable that the plants affected contained less water or had more thickened anatomical parts than the normal plants, as is the case in some pathological conditions. The corn grown in the higher aluminum concentrations probably was competing more for water than were normal plants.

MIXTURES OF ALUMINUM CITRATE AND CALCIUM HYDROXIDE.—In growing plants in mixtures of the salts of the more electro-positive elements, the relative effects are more easily shown, for the growth effects are for the most part those produced by the cations. When mixtures containing aluminum salts are concerned a more complex relationship is introduced, owing to the secondary products of hydrolysis. Thus in the solutions of all aluminum salts

TABLE IV

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	pH
66.4	53.8	68.8	0	6.61
96.1	82.1	89.5	2	6.67
94.1	82.1	95.9	5	6.37
99.9	97.0	97.8	15	6.30
92.3	97.8	97.4	30	6.25
78.9	85.8	76.7	50	6.25
83.0	90.7	83.3	70	6.14
87.8	93.1	91.9	85	5.95
81.1	84.3	88.6	95	5.95
78.0	93.4	83.6	98	5.95
75.5	78.1	77.0	100	5.51

TABLE V

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	%	pH
75.5	78.1	77.0	0	100	5.07
88.7	48.2	71.3	2	98	4.33
77.1	76.1	70.8	5	95	4.05
55.7	65.7	62.8	15	85	3.85
62.4	63.9	70.5	30	70	3.68
45.9	46.6	54.0	50	50	3.66
32.2	41.6	57.9	70	30	3.50
20.2	35.2	57.5	85	15	3.50
10.3	17.7	40.8	95	5	3.50
3.6	12.4	30.3	98	2	3.50
7.2	13.4	34.9	100	0	3.51

the cation gives rise to hydrogen ions in measurable quantities. We are not dealing with two elements, which are cations, but in addition the cation hydrogen ions in excess of hydroxyl anions. The influence of the additional ions renders interpretation of relative growth data more difficult. In this investigation growth data were compared when corn seedlings were grown in aluminum citrate alone, in calcium hydroxide, and in mixtures of the two compounds. The total molecular concentration was not changed, 0.006 M of calcium hydroxide and aluminum citrate.

In order to express more adequately the effects of these compounds when

TABLE VI

GROWTH OF CORN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + POTASSIUM HYDROXIDE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M POTASSIUM HYDROXIDE	REACTION
%	%	%	%	%	pH
13.6	52.9	79.8	0	100	9.8
31.7	52.3	77.8	2	98	9.7
16.7	36.1	65.8	5	95	9.7
18.5	38.4	74.5	15	85	9.6
25.2	49.0	79.1	30	70	9.0
73.8	69.6	94.3	50	50	8.36
32.0	43.5	68.0	70	30	6.00
14.3	36.5	70.4	85	15	4.15
11.9	29.2	67.1	95	5	3.84
12.1	25.6	64.1	98	2	3.45
7.2	13.4	34.9	100	0	3.43

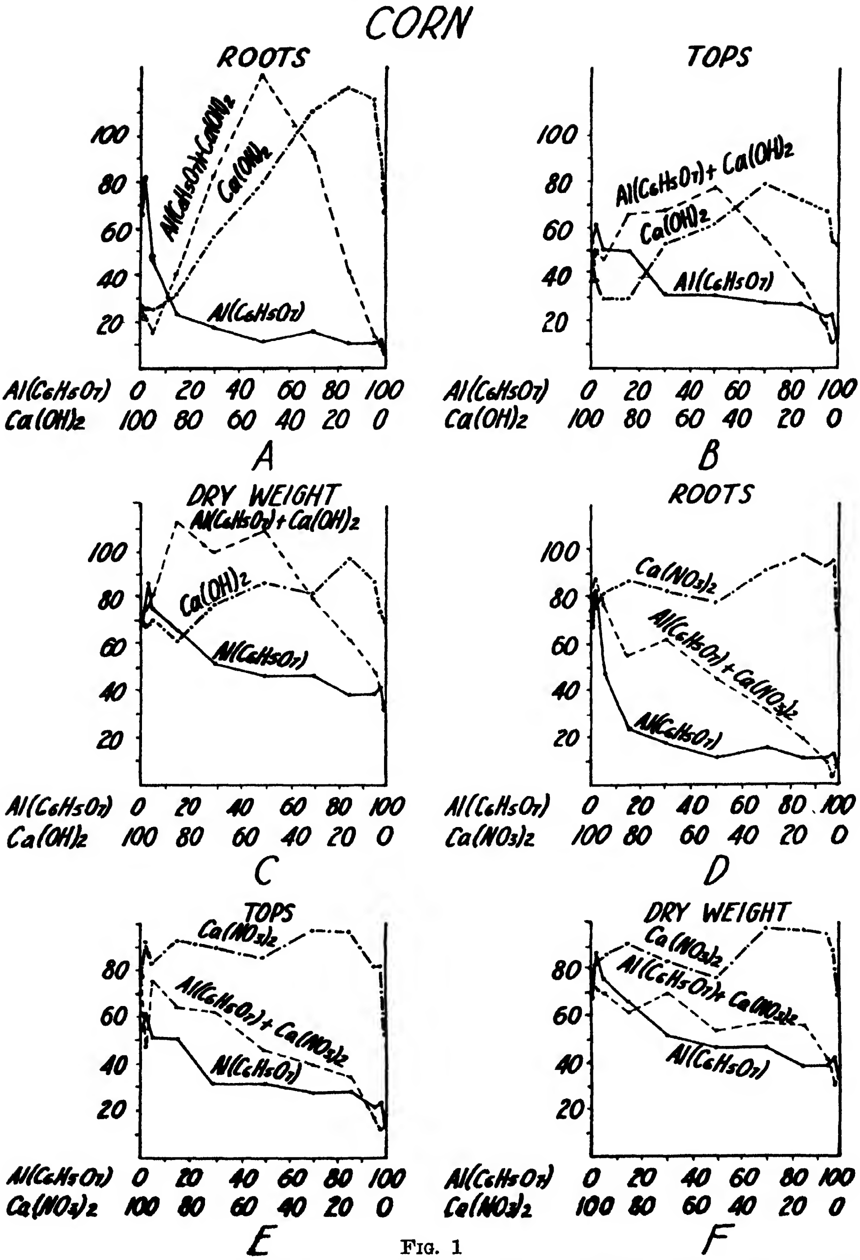

FIG. 1

A, B, C, D, E, F: Elongation of corn roots and tops together with total dry weight in simple solutions and mixtures of aluminum citrate and calcium hydroxide, also in simple solutions and mixtures of aluminum citrate and calcium nitrate. Ordinates represent percentages of elongation and total dry weight; abscissas represent percentage molecular proportions of 0.006 M.

in mixture, it is essential to know what is the effect of calcium hydroxide when used singly (see table II). The molarity was based on 0.006 M calcium hydroxide. The curve (fig. 1 *A*) would indicate that low concentrations of calcium hydroxide are stimulating to growth but that at higher concentrations the relative growth curve rapidly falls for the relative root length, and to a lesser degree for tops and total dry weight. The deleterious effect is not to a great extent due to the calcium ion, but rather to the hydroxyl ion. For all cases the range of toxicity is greater for the aluminum salt than for the calcium base (fig. 1 *A, B, C*).

One of the most outstanding characteristics of growth relationships is the abnormal stimulation to growth of roots in the seedling stage when the plants are grown in mixtures of aluminum citrate and calcium hydroxide (table III). In part this is due to the neutralization of the acidic properties of the aluminum compounds. The neutralization is not the cause of the increased growth, however, for roots and total dry weight are greater than the growth results with either the aluminum salt or the calcium base. This reaction does not confine itself to single groups, but applies to all plants used in the experiment, which represent the mean of thousands of plants. It would seem that a neutral salt would indicate the effect of calcium ion without the accompanying neutralization of the hydroxyl ion.

It may be stated that the combined action of the hydroxyl ion and the calcium ion results in a marked effect in overcoming the toxicity of the aluminum and the hydrogen ions in growth trials.

GROWTH RESULTS OF MIXTURES OF CALCIUM NITRATE AND ALUMINUM CITRATE.—In single salt solutions one of the salts least toxic to seedlings is calcium nitrate in dilute solutions (**8**). The low toxicity makes it difficult to secure consistent growth results (table IV). The growth of corn seedlings in varying percentage proportions of 0.006 M was not greatly reduced from that of the controls. It does not modify to any degree the hydrogen ion concentration of a medium in which it is placed. When various molecular proportions of calcium nitrate and aluminum citrate are mixed, the results indicate that the aluminum ions and hydrogen ions increase the toxicity of the solution as compared with the results obtained from the calcium nitrate salt used singly, but that the calcium salt ameliorates the toxic effects of the aluminum ion. It can definitely be stated that the calcium ion exerts an antagonistic action on the aluminum ion as concerns growth promotion. As is shown by the relative growth promoted by the addition of calcium and hydroxyl ion formed, however, the nearly neutral salt in no instance has the extreme action that is seen when calcium oxide is used instead of the nitrate (fig. 1 *D, F* and table V).

ALUMINUM CITRATE AND POTASSIUM HYDROXIDE.—Potassium hydroxide was used in mixtures with aluminum citrate to determine to what extent it

TABLE VII

Growth of corn seedlings in solutions of citric acid equivalent in acidity to corresponding aluminum salt

Roots	Tops	Dry weight	Equivalent in pH of aluminum citrate percentage 0.006 M	Reaction
%	%	%	*pH*	*pH*
66.4	53.9	68.8	0	5.82
72.3	68.6	77.8	2	4.75
89.0	62.4	65.4	5	4.50
72.2	75.6	83.3	15	4.50
70.1	81.6	93.1	30	4.50
51.4	66.5	78.0	50	4.05
34.1	39.9	70.7	70	3.64
25.0	45.5	57.3	85	3.55
4.5	25.2	45.4	95	3.55
15.7	42.2	60.9	98	3.50
10.2	41.7	54.8	100	3.43

would resemble calcium hydroxide in growth promoting properties when mixed with the same aluminum salt. In no instance did it cause the abnormally large growth which occurred with the calcium base. There was low toxicity over a wide range of concentration of the mixture as shown by total dry weight. For roots and tops a point was attained where marked improvement occurred, 50 per cent. of 0.006 M of each component. On one side of this point the hydrogen and aluminum ions were apparently too

TABLE VIII

Growth of soy bean seedlings in solutions of aluminum tartrate + potassium hydroxide

Roots	Tops	Dry weight	Percentage 0.006 M aluminum citrate	Percentage 0.006 M potassium hydroxide	Reaction
%	%	%	%	%	*pH*
16.9	38.1	49.4	0	100	9.8
8.6	34.7	47.8	2	98	9.4
18.2	38.5	50.8	5	95	9.2
10.1	41.4	65.8	15	85	9.0
67.8	64.4	62.4	30	70	9.0
14.2	28.9	47.2	50	50	7.66
6.0	17.8	36.9	70	30	5.30
3.0	13.9	36.4	85	15	4.15
4.3	11.0	29.3	95	5	3.85
1.4	11.8	26.2	98	2	3.56
4.8	18.0	31.4	100	0	3.45

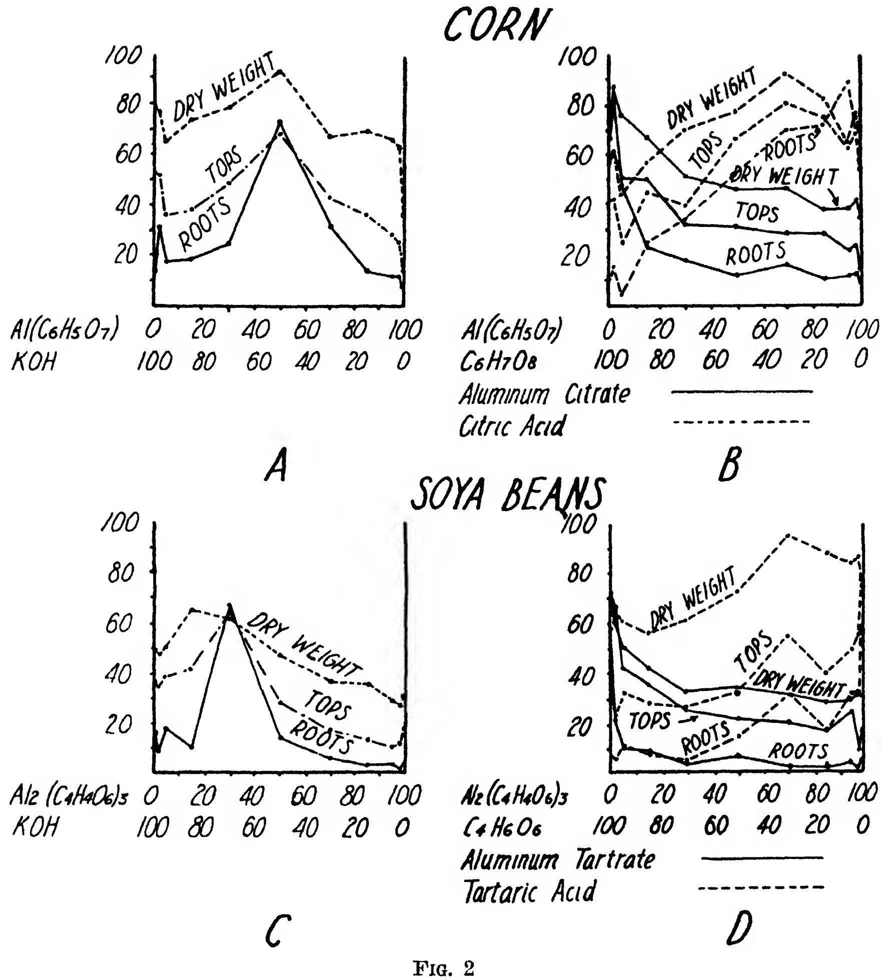

FIG. 2

A: Elongation of corn roots and tops together with total dry weight of corn seedlings grown in simple and mixed solutions of aluminum citrate and potassium hydroxide.

B: Elongation of corn roots and tops in addition to total dry weight of seedlings grown in aluminum citrate, and in citric acid, the latter of sufficient concentration to make the acid solution of pH equal to the corresponding aluminum salt solutions.

C: Elongation of soy bean roots and tops in addition to dry weight of seedlings grown in simple and mixed solutions of aluminum tartrate and potassium hydroxide.

D: Elongation of soy bean roots and tops in addition to total dry weight of seedlings grown in aluminum tartrate and tartaric acid. Concentration of the acid was sufficient to make it of pH equal to the corresponding salt solutions.

active, with little antagonism of the potassium ion toward the aluminum; and on the other side the alkalinity was too pronounced. The general outline of the curve would suggest a point of neutralization for best relative

growth with meager action of potassium in retarding the influence of aluminum (fig. 2 *A* and table VI).

COMPARATIVE EFFECT OF ALUMINUM CITRATE AND CORRESPONDING CITRIC ACID.—Some controversy has arisen regarding the extent to which the hydrogen ion causes the deleterious effects in aluminum toxicity experiments. In order to make an approximate comparison, corn seedlings were grown in water solutions that were made acid with citric acid to the same extent as were the corresponding aluminum citrate solutions. This was done by comparing indicator reactions. In the extremes of low concentration the growth was not very different. As the concentration of the salt and the acid increased there was a gradual fall in the relative growth. The fall in the growth curve (fig. 2 *B*) is more rapid for the aluminum salt than for the corresponding acid. Thus at 50 per cent. of 0.006 M, the relative growth value of corn roots for aluminum salt is approximately 12 as compared with 51 for the acid (see table VII). At 30 per cent. of the same total concentration for the total aluminum salt concentration on corn roots the value is about 18, and for the acid, 70. This would suggest that the toxicity of aluminum is always augmented by hydrogen ions, but the relative effects are not nearly of equal value. It may be stated that much of the actual toxicity of aluminum salts is due to the aluminum ion.

SOY BEANS

Soy beans were grown during the seedling stage in various percentage proportions of 0.006 M aluminum tartrate and citrate. Aluminum tartrate contains proportionately twice as much aluminum as does the same molarity of aluminum citrate. Tartaric acid is a dibasic acid, while the citrate is a salt of a tribasic acid.

ROOTS.—At a concentration of 5 per cent. of 0.006 M aluminum tartrate, the toxicity toward the root system is nearly equal to the maximum, equivalent to about 16 p.p.m. of aluminum.

TOPS AND TOTAL DRY WEIGHT.—The maximum toxicity as indicated by tops and total dry weight is not attained until the plants are grown in a concentration equal to 30 per cent. of 0.006 M. This is only slightly less than 100 p.p.m. (fig. 3 *A, B, C*). At this concentration and higher the roots seemingly did not grow at all.

As with corn, the soy beans were grown in solutions containing fractional parts of the total molecular concentration, 0.006 M calcium hydroxide. At moderately low concentrations there is a marked stimulation to growth in the seedling stage. This increase is measurably greater than that of the control. Although soy beans have been classified as of low lime requirement (**33**), concentrations of calcium hydroxide equivalent to 0.0018 M of calcium appear to augment growth above the control for roots of the beans.

TABLE IX

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF TARTARIC ACID

ROOTS	TOPS	DRY WEIGHT	TARTARIC ACID EQUIVALENT TO pH OF CORRESPONDING PERCENTAGE OF ALUMINUM SALT	REACTION
%	%	%	%	*pH*
59.6	73.3	76.8	0	6.61
32.8	55.5	87.4	2	4.45
32.4	49.4	84.5	5	4.44
17.8	40.1	88.9	15	4.25
32.1	55.7	95.8	30	3.80
15.5	33.0	73.2	50	3.62
5.9	27.5	61.4	70	3.54
8.3	29.7	56.2	85	3.54
11.9	33.6	61.3	95	3.45
6.9	24.6	65.8	98	3.49
7.5	38.8	66.8	100	3.45

Above this concentration the growth of roots falls rapidly. The various concentrations of calcium oxide do not materially affect the relative growth of tops and total dry weight. The lowest concentration is not appreciably different in affecting growth promotion from that of the highest concentration.

MIXTURES OF ALUMINUM TARTRATE AND CALCIUM HYDROXIDE.—When the aluminum salt and the calcium base are mixed in various proportions the growth stimulation properties are changed. The marked relative growth is indicative when the proportions of 0.006 M are 30 per cent. of the aluminum salt and 70 per cent. of the base. The pH of the solution promoting maximum

TABLE X

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM TARTRATE	REACTION
%	%	%	%	*pH*
59.7	73.3	76.8	0	6.61
21.4	66.7	61.6	2	4.75
10.9	42.5	50.9	5	4.44
9.8	36.8	52.9	15	4.25
4.5	26.9	33.2	30	3.80
6.6	22.5	34.8	50	3.62
0.4	20.9	32.2	70	3.54
0.5	17.5	29.0	85	3.54
3.0	25.0	29.5	95	3.45
0.5	9.9	31.9	98	3.45
4.8	18.0	31.4	100	3.45

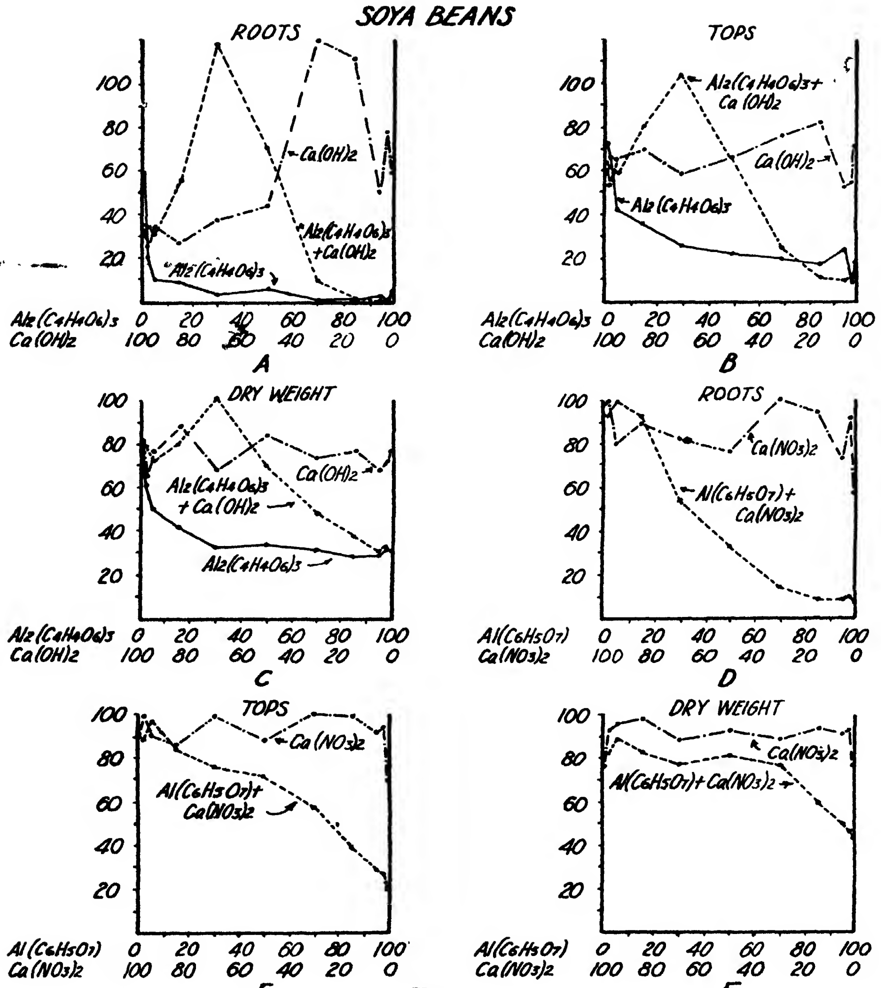

FIG. 3

A, B: Elongation of roots and tops of soy beans grown in simple and mixed solutions of aluminum tartrate and calcium hydroxide. Ordinates represent percentages of elongation for standard solution; abscissas represent percentage molecular proportions of 0.006 M.

C: Dry weight of soy beans grown in simple solutions and mixture of aluminum tartrate and calcium hydroxide.

D: Elongation of roots of soy beans grown in simple salt solution and mixtures of aluminum citrate and calcium nitrate.

E: Elongation of tops of soy beans grown in single salt solutions and in mixtures of aluminum tartrate and calcium nitrate.

F: Dry weight of soy beans grown in single salt solutions and mixtures of aluminum tartrate and calcium nitrate. Ordinates represent percentages of elongation and total dry weight; abscissas represent percentage molecular proportions of 0.006 M.

TABLE XI

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF CALCIUM HYDROXIDE

Roots	Tops	Dry weight	Percentage 0.006 M calcium hydroxide	Reaction
%	%	%	%	*pH*
59.7	73.3	76.8	0	6.61
78.7	55.5	73.0	2	7.85
50.6	53.2	67.6	5	7.85
111.2	83.0	77.3	15	8.10
120.0	76.9	74.6	30	9.7
44.2	66.8	84.3	50	9.7
38.3	59.1	67.5	70	9.7
27.6	70.1	88.7	85	9.8
35.1	66.4	76.8	95	9.8
24.2	53.5	64.6	98	Λ
36.5	64.7	82.5	100	Λ

growth in single calcium hydroxide solutions was slightly above 9 (table XI). The pH of the mixture solution yielding best relative growth was about 6.5 (table XII). The beneficial effects of the mixture were in part due to calcium ion with an accompanying optimum pH for this particular concentration. The abrupt drop in growth curve suggests neutralization. It might be added that calcium hydroxide and aluminum salts are the only mixtures used that yield results in which the stimulation to growth in some instances is higher than that of the controls. The writer has not a positive explanation as of behavior. The comparison is made with mixtures of aluminum salt and cal-

TABLE XII

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE + CALCIUM HYDROXIDE

Roots	Tops	Dry weight	Percentage 0.006 M aluminum tartrate	Percentage 0.006 M calcium hydroxide	Reaction
%	%	%	%	%	*pH*
36.6	64.7	82.5	0	100	. .
35.3	62.2	79.9	2	98	.. ..
31.4	59.4	72.8	5	95	
54.1	81.1	80.1	15	85	7.65
118.1	104.3	100.8	30	70	6.50
71.5	65.4	70.3	50	50	5.66
10.3	25.4	48.9	70	30	4.18
1.2	11.8	38.6	85	15	3.70
0.0	10.0	31.0	95	5	3.60
1.3	12.0	33.5	98	2	3.60
4.8	18.0	31.3	100	0	3.45

TABLE XIII

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	*pH*
59.7	73.3	76.8	0	6.61
92.5	94.8	92.8	2	6.65
74.0	92.5	91.0	5	6.37
95.0	99.8	93.4	15	6.30
100.2	100.7	89.0	30	6.25
76.7	88.4	92.7	50	6.14
82.8	99.4	88.3	70	5.95
89.4	85.3	97.0	85	5.95
80.1	90.1	95.9	95	5.95
99.3	99.5	92.9	98	5.95
98.6	90.7	76.0	100	5.51

cium nitrate, and mixtures of aluminum salt and potassium hydroxide. This would suggest that an expression of pH is more significant when stated in relationship to other elements than as an entity itself.

MIXTURES OF ALUMINUM CITRATE AND CALCIUM NITRATE.—The toxicity of calcium nitrate toward soy bean seedlings is only slight, approximately like that of the same salt toward corn (table XIII).

Mixtures of calcium nitrate and aluminum citrate are more indicative of an approximate mean of the toxicity of calcium nitrate and aluminum citrate each used singly. Another trial of a series of concentrations with

TABLE XIV

GROWTH OF SOY BEAN SEEDLINGS IN SOLUTIONS OF ALUMINUM CITRATE + CALCIUM NITRATE

ROOTS	TOPS	DRY WEIGHT	PERCENTAGE 0.006 M ALUMINUM CITRATE	PERCENTAGE 0.006 M CALCIUM NITRATE	REACTION
%	%	%	%	%	*pH*
93.0	90.7	81.2	0	100	5.07
92.8	87.9	81.9	2	98	4.33
99.6	96.4	88.8	5	95	4.05
92.3	84.0	82.6	15	85	3.85
54.0	75.8	77.3	30	70	3.68
33.2	71.9	81.0	50	50	3.66
14.8	57.8	76.7	70	30	3.50
8.7	39.3	59.5	85	15	3.50
8.6	28.6	50.1	95	5	3.50
9.1	26.9	46.8	98	2	3.50
8.1	19.7	43.8	100	0	3.51

TABLE XV

GROWTH OF BUCKWHEAT SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE

Roots	Tops	Dry weight	Percentage 0.006 M aluminum tartrate	Reaction
%	%	%	%	pH
5.7	13.5	29.7	0	6.61
9.0	13.5	25.1	2	4.75
3.7	11.3	29.9	5	4.44
3.1	10.1	33.7	15	4.25
15.1	15.2	56.3	30	3.80
13.4	12.0	61.3	50	3.62
14.8	14.3	53.7	70	3.54
12.1	10.4	52.3	85	3.54
14.3	10.7	31.6	95	3.45
5.3	9.4	39.4	98	3.49
11.5	8.1	20.0	100	3.45

aluminum citrate used singly would have lent itself to a fuller explanation of this action. The two growth curves, however, that of calcium nitrate and that of aluminum citrate and calcium nitrate, are not greatly different from those when corn was used; it thus seems a reasonable conclusion that the growth curve of the single aluminum salt would not be greatly different from that for corn (table XIV).

The calcium ion effect on total dry weight suggests a definite detoxifying effect. As in corn, this is more pronounced for relative total dry weight and relative length of top than for roots (fig. 3 *D, E, F*).

TABLE XVI

GROWTH OF BUCKWHEAT SEEDLINGS IN SOLUTIONS OF ALUMINUM TARTRATE + CALCIUM HYDROXIDE

Roots	Tops	Dry weight	Percentage 0.006 M aluminum tartrate	Percentage 0.006 M calcium hydroxide	Reaction
%	%	%	%	%	pH
127.9	70.9	93.7	0	100	V
177.5	73.6	94.0	2	98	9.0
194.7	66.6	86.6	5	95	8.0
168.3	66.4	77.4	15	85	7.65
81.0	52.8	90.1	30	70	6.50
21.9	17.9	43.2	50	50	5.60
22.2	10.2	24.3	70	30	4.18
16.7	11.4	21.1	85	15	3.70
17.7	8.1	16.1	95	5	3.40
16.2	7.4	20.5	98	2	3.40
11.5	8.1	20.0	100	0	3.40

Mixtures of aluminum tartrate and potassium hydroxide.—To determine the effect of the different hydrogen ion concentrations, bean plants were grown in mixtures of the two compounds aluminum tartrate and potassium hydroxide. As with corn, there seemed to be a rather definite point for favorable growth promotion. The potassium ion here as before did not seem to overcome the toxicity to any great extent; but at one concentration, 30 per cent. aluminum tartrate, 0.006 M, and 70 per cent. potassium hydroxide, 0.006 M, there was a fair medium for growth promotion. It is the writer's opinion that it is more of an index of favorable hydrogen ion concentration than a favorable mixture of the other cations, aluminum and potassium. The pH for the best growth in this trial was between 8 and 9 (table VIII). This was approximately the same as for corn in the aluminum citrate and potassium hydroxide mixture.

TABLE XVII

Growth of buckwheat seedlings in solutions of aluminum tartrate + potassium hydroxide

Roots	Tops	Dry weight	Percentage 0.006 M aluminum tartrate	Percentage 0.006 M potassium hydroxide	Reaction
%	%	%	%	%	*pH*
1.6	9.2	24.9	0	100	9.6
2.8	9.4	28.4	2	98	9.4
3.1	10.3	26.7	5	95	9.2
16.4	13.8	31.2	15	85	9.0
87.6	78.7	71.5	30	70	9.0
13.7	37.2	44.4	50	50	7.66
4.3	13.7	24.6	70	30	5.30
4.0	10.1	23.5	85	15	4.15
3.1	6.5	12.0	95	5	3.84
3.5	6.5	10.7	98	2	3.56
11.7	8.1	20.0	100	0	3.45

It is worthy of note that the seemingly optimum pH of solutions for the seedling stage is not like that of most soils for yielding crops. The optima are higher for solutions. For permanent growth in soils at high pH much of the common vegetation of a humid region would not thrive so well as at a lower pH. This may be due to precipitation and complete isolation of some required element of the field, or it may be possible that the hydroxyl ion only slowly permeates the protoplasm of the seed and does not disturb the equilibrium during the short time the seedling gets its nutrients from the seed.

Comparison of toxicity of aluminum tartrate and tartaric acid.—Water solutions were made acid with tartaric acid, comparable to the acidity

of the corresponding aluminum salt, aluminum tartrate, at different concentrations and soy bean seedlings were grown in the solution. This was a similar procedure to that adopted for corn. The results are as with corn in citric acid. The aluminum salt solutions with hydrogen ion concentrations the same as those of the corresponding acid caused lower relative growth rates than did the acid. This was the case for roots, tops, and total dry weight (fig. 2 *D* and tables IX and X).

Buckwheat

Corn and soy beans bore considerable resemblance in their reaction to aluminum salts. Buckwheat in certain respects demonstrates a different behavior. This plant in the seedling stage is seemingly more sensitive to distilled water-low osmotic pressures. This was indicated by the results of several trials, each trial including 50 seedlings. Also for single salt cultures of aluminum tartrate there is little difference between the comparative lengths of tops and roots (table XV). The plant seems to be more sensitive to low concentrations than it is to moderate concentrations of aluminum tartrate. At 0.0018–0.003 M the relative growth is better for roots, tops, and total dry weight than it is at lower and higher concentrations of the same salt. In other experiments the relative root length was the most pronounced with respect to retardation of relative growth. In the case of buckwheat the relative top length is almost equally affected. The total dry weight is higher, suggesting low water content or thickening and branching of stems and roots.

Mixtures of aluminum tartrate and calcium hydroxide.—Relative growth of buckwheat is promoted by the higher concentrations of lime rather than by the lower concentrations. The root system is most markedly affected by high proportions of calcium hydroxide (approximately 95 per cent. 0.006 M calcium oxide and 5 per cent. 0.006 M aluminum tartrate). This effect diminishes rapidly with lower lime nutrients, until at the point at which the proportions are 50 per cent. of 0.006 M lime and 50 per cent. of 0.006 M aluminum tartrate the results are almost the same with respect to relative growth as if no lime were added at all (table XVI). The same behavior applies, only to a more limited degree, to the case of relative length of tops. With respect to total dry weight the mixture finally indicates over a range a condition of apparent additive effect with respect to toxicity, the mixture promoting a smaller relative growth than the single toxic salt (fig. 4 *A, B, C*).

Mixtures of aluminum tartrate and potassium hydroxide.—When potassium hydroxide is mixed with aluminum citrate in different proportions the growth is retarded markedly except in one proportion. This proportion is approximately 30 per cent. of 0.006 M aluminum tartrate and 70

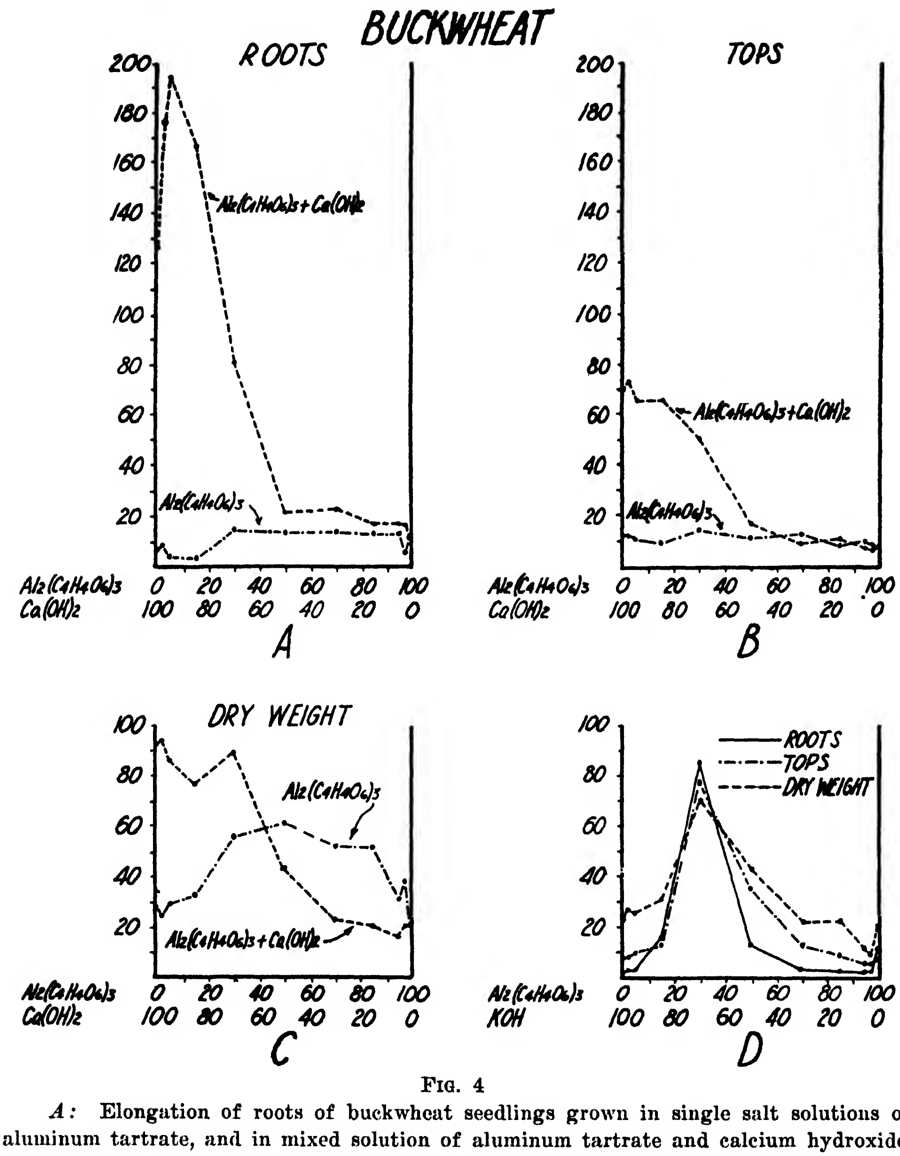

FIG. 4

A: Elongation of roots of buckwheat seedlings grown in single salt solutions of aluminum tartrate, and in mixed solution of aluminum tartrate and calcium hydroxide.

B: Elongation of tops of buckwheat seedlings grown in single salt solutions of aluminum tartrate, and in mixed solution of aluminum tartrate and calcium hydroxide.

C: Total dry weight of buckwheat seedlings grown in single salt solutions of aluminum tartrate and in mixed solutions of aluminum tartrate and calcium hydroxide.

D: Elongation of roots and tops in addition to total dry weight of buckwheat seedlings grown in mixtures of aluminum tartrate and potassium hydroxide. Ordinates represent percentages of elongation and total dry weight; abscissas represent percentage molecular proportions of 0.006 M.

per cent. 0.006 M potassium hydroxide. In all others the growth falls off markedly. For seedlings growing in water solutions this probably indicates an optimum pH. The potassium evidently does not have much effect in alleviating the toxicity of aluminum, and at the higher pH the condition is too alkaline for the plants to thrive. The optimum pH is almost 9 (table XVII). This optimum does not nearly approach the optimum for mixtures of the aluminum salt and lime with respect to the degree of relative growth (fig. 4 *D*). Mixtures of lime and aluminum salts for all plants investigated have wide and high limits of growth, while potassium mixtures at nearly the same pH have narrower and lower limits of relative growth.

Summary

1. Organic aluminum compounds exert a distinct toxic effect on the plants of corn, soy beans, and buckwheat. The toxicity increases with increased concentration.

2. The toxicity for the three plants could be most effectively overcome by calcium hydroxide. Calcium nitrate could be used as an antagonist of aluminum, but not as effectively as the calcium base.

3. Potassium hydroxide was less effective in counteracting the toxicity of aluminum than was calcium hydroxide. It apparently served to neutralize the acidity of the aluminum salt.

4. The favorable hydrogen ion concentration is not the same for all solutions. The optimum pH varies with the components and their proportions present in the solution.

5. Comparing the hydrogen ion concentration of the organic acids with similar hydrogen ion concentrations of the corresponding aluminum salts of these acids, it is shown that at appreciable concentrations the aluminum salt suppresses growth to a markedly greater extent than does the acid.

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LINKAGE BETWEEN OUTPUT OF ELECTRIC ENERGY BY POLAR TISSUES AND CELL OXIDATION

H. F. Rosene and E. J. Lund

(with six figures)

Introduction

Evidence is presented in this report which it is believed establishes the following facts: (*a*) A quantitative relation exists between cell oxidation and the continuously maintained E.M.F.'s in the root of the onion, ***Allium cepa***. (*b*) Equal change in oxygen concentration around different regions of the root tip produces an unequal change in E.M.F.; in this way the characteristic distribution of E.M.F.'s per unit length of root and therefore the pattern of the correlation currents in the root are modified in a characteristic manner by change in oxygen tension. (*c*) The velocity of oxygen consumption per unit volume of tissue is greatest in the region of active cell division, and the velocity of oxidation in this region is also changed to a greater degree by the same change in oxygen tension than in other regions of the root tip. This fact, which appears to have an important bearing upon the nature of bioelectric currents, was previously discovered in the *Obelia* stem (**17**). The experiments supply the connecting link in the evidence for the validity of the theory that the continuously maintained E.M.F. in a polar cell is that of a redox system maintained in state of flux equilibrium as defined elsewhere by one of us (**17**).

There is a correspondence of morphological, functional, and electrical polarity along the root axis of *A. cepa*. In the unstimulated uninjured condition, the region of active cell division is invariably electropositive in the external circuit to more basal regions; and the distribution of potentials is in general characteristic but in detail specific for each root. For illustrations of the exact distribution of these potentials the reader is referred to the curves in the paper by Lund and Kenyon (**13**). Under constant external conditions, spontaneous fluctuations in electric polarity occur and the distribution of E.M.F. per unit length varies from time to time (**20**). The first evidence that cell respiration and electric potentials on the root are linked phenomena was furnished by Lund and Kenyon (**13**). They found that the greatest reduction of methylene blue per unit length occurs in the region of high positive potential, where active cell division takes place, and the same region also produces more CO_2 per unit length. A corresponding distribution of the concentration of sulphydryl groups per unit length has been demonstrated; the region of active cell division exhibits the most intense color when the nitroprusside test is applied (**17**).

The present investigation was limited to experiments on not more than 7 mm. of the distal end of the root and for the most part to 6 mm. or less. This region of 6 ± mm. will be designated the "root-apex" to facilitate presentation. The occurrence of progressive cellular and tissue differentiation from primordial meristematic cells to permanent tissue is a salient feature of the polar structure of the root-apex. In roots 40 to 65 mm. in length, the root cap is not over 0.35 mm.; the region of active cell division, 1 to 1.5 mm.; and the zone of cell enlargement and cell maturation, 1 to 2 mm. Permanent tissues are present in the fifth and sixth millimeters. In recent detailed experiments performed in this laboratory by A. H. HANSZEN, it has been observed that the greatest amount of elongation takes place within the third millimeter. Although the transitional stages are not definitely delimited, the cells in the distal third (2 ± mm.) of the root-apex are relatively young, those in the middle third (2 ± mm.) are approaching maturity, and those in the proximal third (2 ± mm.) are relatively old. In this paper attention will be focused upon the electrical behavior of these three regions in relation to concentration of oxygen.

Methods

Since the material used in the experiments involved only a total of 6 or 7 mm. of the root tip, and since the conditions for the experiment often demanded that oxygen or other gases be accurately applied to a root region 1 mm. in length, without mechanical stimulation and any apparent change in humidity or temperature, a new apparatus and technique was developed. This has shown itself to be an appreciable improvement over other procedures. The apparatus is susceptible of modification for particular purposes and has been found so satisfactory that a detailed description seems justified. Recent adaptations of the apparatus permit very precise procedures of manipulation and measurements on certain single cells under ideal conditions.

The electrode chamber is illustrated in figure 1. A removable glass cover (*H*) fits closely to a rubber cushion (*P*), which is cemented to a block of bakelite (*C*) that serves as the floor of the chamber. The base (*C*) has four large and four small perforations through which connections penetrate to the outside. A rod (*F*) which passes through one of the large perforations in the rear is attached to a short piece of glass tubing that insulates and serves as a support for the adjustable swivel attachment (*K*). The onion bulb is held and protected by a paraffined cork ring which fits a hole in this attachment. The other opening (*M*) in the rear of the base furnishes means by which additional apparatus (fig. 2, B) may be inserted and connected to the outside for manipulation. Capillary tubes (*T* and *T'*), attached to electrode cups (*D* and *D'*), pass through the large open-

ings in front. The four large perforations are lined by rubber finger-stalls (N) which keep the chamber airtight and through which the movable connections penetrate. Leads from isoelectric zinc-amalgam zinc-sulphate electrodes (E and E') pass through insulated glass tubes in two of the small perforations in the base, and glass tubes (I and O) which line the other two provide for the inlet and outlet of gases from external sources. During each experiment the chamber is lined with wet filter paper and a film of water is maintained at its base. The atmosphere of the chamber can be brought to a necessary complete water saturation and can be completely isolated from the exterior.

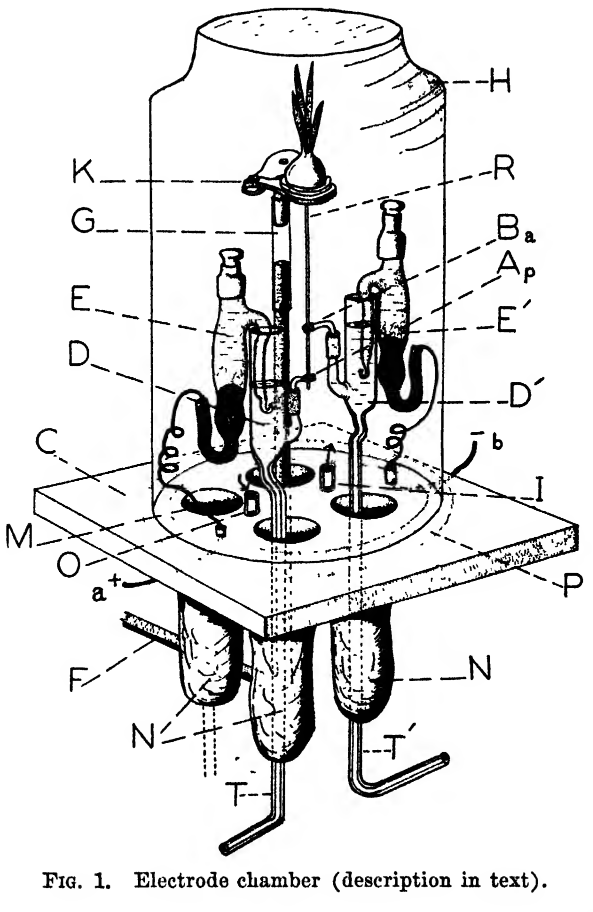

FIG. 1. Electrode chamber (description in text).

The connections outside of the electrode chamber are illustrated in figure 2, A. The bakelite base is firmly attached to a rack and pinion stand (T). Three micromanipulators (A, B, C), each of which has a three-way movement, provide adjustable support for the root holder (K) and the two

electrode cups (D and D', fig. 1). These electrode cups are filled respectively from the connecting reservoirs (R_1 and R_2) through corresponding three-way stopcocks (S_1 and S_2) which are also connected to tubes for draining and washing the cups. Gases may be introduced into the electrode chamber by connecting the inlet tube (I) to the appropriate stopcock (S_3). They may be stored in the reservoirs (E_1 and E_2) of about 75 cc. capacity which are connected to the mercury leveling cups (G_1 and G_2) and passed into the electrode chamber by adjusting the height of the cups, each of which is supported by a rack and pinion stand not illustrated in figure 2; or by appropriate manipulation of the connecting three-way stopcocks they may be admitted directly into the electrode chamber without being stored in the reservoirs.

Gases are applied to or withheld from a specific region by means of a hollow glass jacket (fig. 2, B). The body of the jacket (V) consists of a short piece of glass tubing 4 mm. in length. Small glass coverslips (O) are cemented to the top and bottom and small holes (I) in each just permit passage of the root (R) without its touching the sides of the holes. Glass capillary tubes (P) sealed to two holes in the side of the jacket provide for the inlet and outlet of the gases and are attached to a glass rod (W) which serves as a support. The jacket is inserted into the electrode chamber through an opening (M, fig. 1) in the base and it is manipulated by an additional three-way micromanipulator not illustrated. By means of this micromanipulator and a horizontal microscope the jacket can be accurately adjusted to any position without stimulating or injuring the root. With the jacket in place around the root (R), the openings (I) at the top and bottom are sealed with a small drop of water or paraffin oil. In this way the segment of the root inclosed by the jacket is completely isolated from the atmosphere in the electrode chamber and gases can be introduced into and removed from the jacket under complete control by connecting the inlet tube of the jacket directly to the proper stopcock (S_3 in fig. 2, A). With the jacket in place, the root can be exposed to a gas by either of two methods: (1) introducing the gas directly into the jacket around a particular segment of the root and not into the electrode chamber; or (2) passing the gas into the electrode chamber and not into the jacket, which in that case is filled with moist air.

Electrode contacts are made by the small glass claw projections (K in fig. 2, B) of the electrode cups which are filled with the medium in which the roots grow. The adjustments of the positions of the contacts, which can be accurately controlled by the micromanipulators, are made under a horizontal microscope which is fitted with an ocular micrometer for measurements. From the preceding description it is obvious that the apparatus permits complete and accurate control of all manipulations and environmental conditions of the root.

All experiments were carried out at room temperature which did not vary more than 0.5° C. throughout any one experiment. The onions were grown in tap water and used when the roots were 40 to 50 mm. in length. A different root was used in each experiment. All roots but one were removed from the bulb, care being taken not to stimulate this root when it was placed in position in the electrode chamber. Potentials were measured with a Compton electrometer. In general, the same procedure was followed in each experiment. When adjustments of the positions of the jacket and the contacts on the root had been made, the preparation was left undisturbed for a short period. Observations were then made of the electric behavior of the root during an initial period of exposure to the vapor-satu-

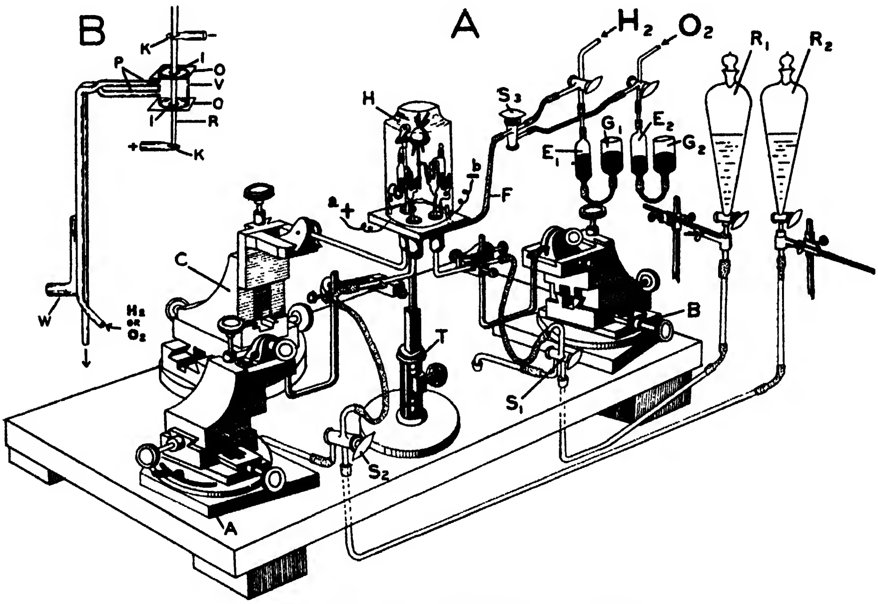

FIG. 2. Apparatus (description in text).

rated atmosphere of the electrode chamber. These readings were continued during the following two successive periods, when (*a*) hydrogen was passed through the electrode chamber or the jacket, and (*b*) oxygen was passed through in a similar manner. Before entering the chamber or the jacket, the hydrogen and oxygen were saturated by passage through distilled water. A uniform and controlled rate of flow of each gas was maintained in all the experiments by suitable pressure gauges attached to the gas tanks. The flow of gas, as such, did not produce any visible effect on the root and did not appear to affect the E.M.F. The root tissue was in no way injured by the experimental procedure and normal growth continued when the roots were replaced in tap water.

Effect on E.M.F. of the root-apex produced by change in oxygen concentration at different regions between electrode contacts

1. Change in E.M.F. when the apical end (1.5 mm.) is exposed to (*A*) hydrogen and (*B*) oxygen.—Figure 3, A shows the position of the jacket and electrode contacts in a series of three duplicate experiments on roots of three different bulbs (curves 1, 2, 3). The basal grounded electrode contact (–) was made at the upper aperture of the jacket and the jacket was filled with moist air.[1] The E.M.F. of the region between the electrodes was allowed to come to a constant value in air during a 10-minute period as shown in curves 1, 2, and 3, figure 3, A. Hydrogen gas saturated with water vapor was then applied to all of the root and bulb (except that segment inclosed by the jacket) through the connecting tubes (*I* and *O*, fig. 1). Since the results of all the experiments were remarkably similar, even in detail, only three curves (1, 2, 3, fig. 3, A) are presented. Each curve shows that the removal of oxygen by hydrogen around the apical 1.5 mm. of the root rapidly diminished the electric polarity of the root included between the electrodes. Curves 2 and 3 show a complete reversal of polarity. The initial large drop in potential was followed by a smaller rise, the magnitude and duration of which was specific for each root. At the end of the period in hydrogen the E.M.F. was lower than at any time when in air. Deprived of available oxygen by the exposure to hydrogen, the apical end of the root-apex did not maintain its characteristic high positive potential and consequently the electric polarity was considerably reduced in magnitude.

Replacement of hydrogen by oxygen saturated with water vapor produced an abrupt rapid increase of the E.M.F. of such great magnitude that the potential difference not only reached but very often exceeded its former value in air, *exhibiting the typical rebound curve observed in experiments on frog skin and Douglas fir* (**15, 18**). Curves 1 and 3, figure 3, A, illustrate the fact that the E.M.F. was often maintained at a new high level in oxygen. The effect of removal of oxygen was promptly and completely reversible.

The curves show that a definite quantitative relationship exists between cell oxidations at the apical end of the root-apex and the magnitude and orientation of electric polarity.

2. Change in E.M.F. when the basal end (1.5 mm.) is exposed to (*A*) hydrogen and (*B*) oxygen.—The experimental arrangement is illustrated in figure 3, B. Four mm. of the root-apex were inclosed by the air-

[1] When both apical and basal contacts were made at the water menisci in the apertures of the air-filled jacket under the same experimental conditions, little or no change in E.M.F. was produced when the part of the root outside of the jacket was subjected to change in oxygen concentration.

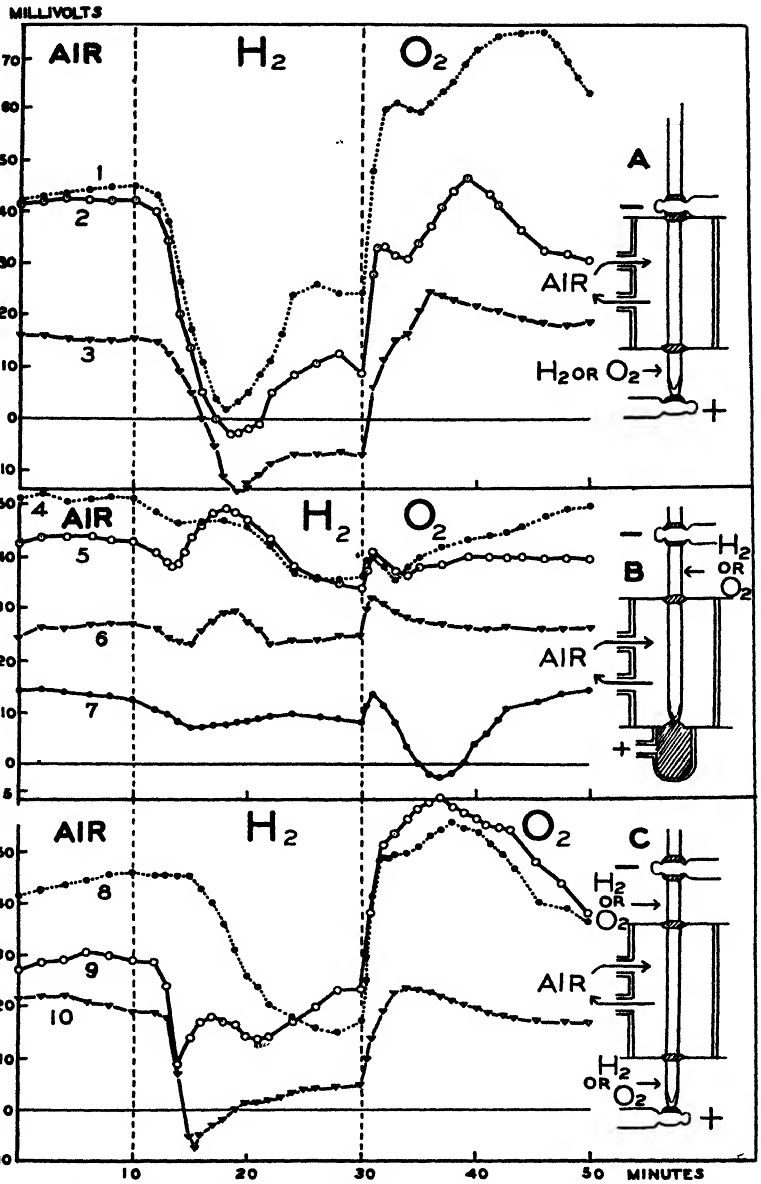

FIG. 3. Effect of change in oxygen concentration at apical and basal ends of root-apex.

A: Effect of (*a*) hydrogen, and (*b*) oxygen at apical end only, on total E.M.F. of root-apex. Curves 1, 2, and 3 obtained from three duplicate experiments on three different roots. Diagram A shows positions of electrode contacts and gas jacket on root-apex.

B: Effect of (*a*) hydrogen, and (*b*) oxygen at basal end only on total E.M.F. of root-apex. Curves 4, 5, 6, and 7 obtained from four duplicate experiments on four different roots. Diagram B shows positions of electrode contacts and gas jacket on root-apex.

C: Simultaneous effect of (*a*) hydrogen, and (*b*) oxygen at both apical and basal ends of the same root-apex on total E.M.F. Curves 8, 9, and 10 obtained from three duplicate experiments on three different roots. Diagram C shows position of electrode contacts and gas jacket on root-apex.

filled jacket and 1.5 mm. at the proximal (basal) end were exposed to change in oxygen concentration. Curves 4, 5, 6, and 7, figure 3, represent typical results and should be compared with curves 1, 2, and 3 of figure 3, A obtained under similar experimental conditions. The only essential difference is that in the two sets of experiments different ends of the root-apex were exposed to the change in oxygen concentration. A comparison of the two sets of curves reveals an unequal effect of equal change in oxygen concentration at apical and basal ends. Passing hydrogen through the electrode chamber when the basal end was exposed did not produce the marked depression of electric polarity which occurred when the apical end was similarly exposed. The electric polarity of the root-apex was never observed to become inverted by an oxygen deficit at the basal end only. Admission of oxygen to the chamber produced a relatively small rebound effect. The relatively small magnitude of this rebound effect in the basal region is comparable to the correspondingly small rebound effects observed in basal regions in the Douglas fir, when the electric polarity is changed by change in temperature (**18, 19**). It should be observed that curves 1, 2, and 3 are quite uniform in appearance. This statement does not apply in the same degree to curves 4, 5, 6, and 7.

If the apical and basal ends of the same root were simultaneously exposed to (*a*) hydrogen and then (*b*) oxygen under the same conditions which obtain in the above sets of experiments, the observed unequal effect of equal change in oxygen concentration on apical and basal ends of different roots should appear. Since it has just been shown that the effect on the apical end was noticeably uniform in character and much greater in magnitude than the effect on the basal end, it would be logical to anticipate results which would give curves similar to curves 1, 2, and 3, figure 3. A small increase in potential difference in hydrogen as shown in curves 5 and 6 and a small decrease in potential in oxygen as illustrated by curve 7 would definitely modify the characteristic form of such curves as 1, 2, and 3 because the principle of summation of cell E.M.F.'s applies in each measurement. When this procedure was followed the expected results were fully realized, as shown in curves 8, 9, and 10 of figure 3, C and what follows.

3. Change in E.M.F. when the apical and basal ends of same root-apex are simultaneously exposed to (*A*) hydrogen and (*B*) oxygen.—Four mm. of the root tip were inclosed by the moist air jacket and 1.5 mm. on either side exposed as shown in the diagram (fig. 3, C). Similar curves were obtained in all the experiments, each of which was obtained from a different root. Only three curves are given, curves 8, 9, and 10, figure 3. They convincingly show the unequal effect on the E.M.F. by equal change in oxygen concentration at apical and at basal ends of the

same root-apex. There is a pronounced similarity between curves 8, 9, and 10, and curves 1, 2, and 3 (fig. 3). Evidently in both sets of experiments the general character of the curves can be attributed to the greater change which took place in the highly electropositive apical end. A temporary complete reversal of polarity is exhibited by curve 10. The conspicuous rebound phenomena observed in oxygen in the first set of experiments reappeared. Its modification in curve 10 may be due to a flattening effect produced by a change in an oppositely oriented E.M.F. in the basal end as exhibited by the dip in curve 7, figure 3. In this connection it is of special theoretical interest to note the uniform appearance of (*a*) the relatively large depression of E.M.F. in hydrogen and (*b*) the conspicuous rebound phenomena in oxygen, whenever the *apical end* was exposed.

4. CHANGE IN E.M.F. WHEN AN INTERMEDIATE REGION BETWEEN APICAL AND BASAL ENDS IS EXPOSED TO CHANGE IN CONCENTRATION OF OXYGEN.—The position of the jacket as shown in figure 4, D is similar to that illustrated in figure 3, C, except that connections were made from the hydrogen and oxygen reservoirs (E_1 and E_2, fig. 2) to the glass jacket around the root. By careful vertical movement of the rack and pinion stand which supported the mercury in the leveling cups (G_1 and G_2, fig. 2), a constant flow of gas through the jacket was maintained at such a rate that the mechanical conditions of gas flow as such produced no effect on the E.M.F. The lengths of the apical and basal segments, which were outside of the jacket, were not equal in all the experiments; they varied from 0.8 to 1.8 mm.

Since a quantitative relationship between changes in oxygen concentration at the apical and basal ends and the E.M.F. of the root-apex was demonstrated above, and since it has been shown by MARSH (**20**) that the principle of summation of cell E.M.F.'s applies to the first 30 mm. of the root tip, it is reasonable to expect a change in E.M.F. with change in oxygen concentration around the intervening zone (fig. 4, D) under conditions corresponding to those which prevailed in the preceding sets of experiments. Furthermore, it is to be expected that the magnitude and direction of effect will depend not only upon the length of the intermediate exposed zone, but also upon its distance from the distal and proximal limits of the root-apex. For example, if the segments X and Y outside the jacket in figure 4, D are relatively short (*i.e.*, 0.5–1 mm.), the magnitude of the effect would be relatively greater than if a longer region at each end were isolated, because some of the apical tissue would be in the intervening zone inclosed by the jacket, and it has already been found that greater effects are produced in the apical cells.

Curves 1, 2, and 3, figure 4, are representative of the results from eighteen experiments on different roots. The corresponding lengths of X

and Y, which were equal for each root, were respectively 1.8, 0.8, and 1.3 mm. Curve 1 shows that the potential difference was steadily drifting to an increase in E.M.F. during the period in air, but in hydrogen this drift was

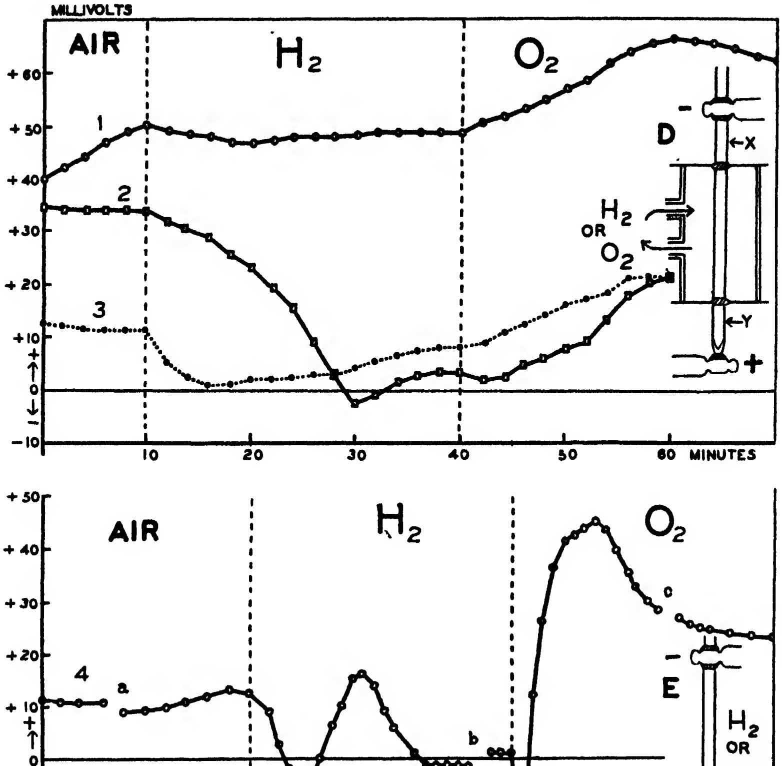

FIG. 4. D: Effect of application of (*a*) hydrogen, and (*b*) oxygen at an intermediate region of root-apex on total E.M.F. Curves 1, 2, and 3 obtained from three similar experiments on three different roots. Positions of electrode contacts and gas jacket shown by diagram D. X and Y are segments of the root exposed to air.

E: Effect of (*a*) hydrogen, and (*b*) oxygen applied simultaneously at all regions of root-apex on total E.M.F. Positions of electrode contacts shown by diagram E. Results shown by curve 4. The breaks (*a*, *b*, and *c*) in curve 4 are the intervals during which the curve of distribution of E.M.F. per unit length of root was determined in (*a*) air, (*b*) hydrogen, and (*c*) oxygen respectively.

opposed in some way and the curve flattened. The drift again manifested itself in oxygen. Curve 2 shows the greatest change. An inverted polarity appeared temporarily in hydrogen and a steady recovery in oxygen. The

relatively greater effects shown in curve 2 are undoubtedly associated with the exposure of a greater portion of the sensitive apical end to change in oxygen concentration. To some extent there is a general similarity of curves 1, 2, and 3, figure 4, to curves 4 and 7, figure 3; but the uniformity of effect on total E.M.F., which is expressed by the relatively large decrease in potential difference in hydrogen and the relatively large increase in oxygen (with its accompanying rebound phenomenon as represented by curves 1, 2, and 3, and curves 8, 9, and 10, in figure 3), is absent. The explanation of the apparently small effect of change in oxygen tension in this set of experiments will be evident from what follows.

Unequal effect on E.M.F. of root-apex produced by simultaneous equal change in oxygen concentration at all regions of root-apex

An inspection of the curves in figures 2 and 3 in LUND and KENYON's paper (**13**) and in figures 3, 4, and 7 of MARSH's paper (**20**), which represent the distribution of E.M.F. over longer regions than the root-apex itself, shows that the occurrence of a *single unidirectional* gradient of E.M.F. is the rule in the length of 6 ± mm. designated in this paper as the root-apex. Many similar determinations of the distribution of potential over the root made by the present writers confirm this conclusion.

The magnitude and form of the curve representing the single gradient in the root-apex are specific for each root. Six such gradients of potential differences in six different roots are illustrated by curves 1a, 2a, 3a, 4a, 5a, and 6a in figure 5. The gradients were determined in the usual manner by moving the electrode contact at the apex by increments of 1 mm. toward the basal contact. In the roots corresponding to curves 4a, 5a, 6a, the basal electrode contact was fixed at a position 6 mm. from the tip; in the roots corresponding to 2a and 3a it was fixed at 5 mm.; and to 1a, at 4 mm. from the tip. Individual variations in the single gradient characteristic of the root-apex are apparent. Curve 5a shows that a point 2 mm. from the tip is electronegative to relatively more basal points, thus producing a small dip in the gradient which indicates the presence of inverted component electric polarities. Similar inverted regions appear in some of the other curves. The form of the gradient would probably appear in more detail if readings were made at intervals of 0.2 mm. instead of 1 mm. The observed gradient of potential of the root-apex is a resultant E.M.F. which expresses the algebraic sum of the E.M.F.'s of individual cells. This does not exclude the possibility of a complex pattern of cellular E.M.F.'s within the root-apex which probably involves series and parallel arrangements of the cells.

The uniformity of results observed whenever the apical end was exposed to changes in oxygen concentration, and the variability of the curves obtained when either the basal end or the intervening region alone was simi-

larly exposed, are related to detailed variations of the distribution of E.M.F. per unit length of the root. A sufficiently large diminution of an inverted component E.M.F. would appear as an increase in the resultant E.M.F. of the gradient. The facts established by the preceding sets of experiments lead to the conclusion that if all the regions of the root-apex were simultaneously exposed to equal change in oxygen concentration, unequal change in regional E.M.F.'s and in the resultant E.M.F. would appear; and furthermore, the generalized nature of the composite curve which would result under these conditions would be determined by the relatively greater changes in apical component E.M.F.'s. Figure 4, E shows the arrangement in a series of experiments of this nature.

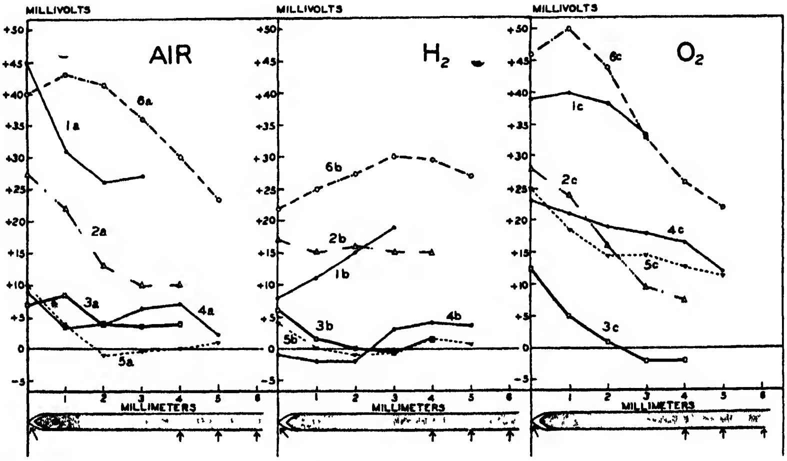

FIG. 5. Effect of change in oxygen concentration on magnitude and form of the curve of distribution of E.M.F. over the length of root-apex in (a) air, (b) hydrogen, and (c) oxygen. The curves were obtained from six experiments on six different roots. Curves 1a, 2a, 3a, 4a, 5a, and 6a, when the electrode chamber (cf. fig. 1) was filled with moist air; curves 1b, 2b, 3b, 4b, 5b, and 6b, when hydrogen was passed continuously through the electrode chamber; and curves 1c, 2c, 3c, 4c, 5c, and 6c, when oxygen was passed continuously through the electrode chamber. The diagrams of the roots below the curves are drawn to scale on the abscissa. Arrows at the apex indicate position of positive electrode. The three arrows at the base indicate the exact positions of the negative electrode which was stationary at different distances from the tip in the different experiments. Corresponding to curves 1a, 2a, and 3a; and 4a, 5a, and 6a, the basal electrode was fixed as shown at 4, 5, and 6 mm. respectively from the tip.

The distance of the basal electrode contact from the contact at the tip was not the same in all the experiments. Hydrogen and oxygen were passed through the electrode chamber. The curves for different roots were essentially alike. Curve 4, figure 4, is typical. In this experiment the electrode contacts were 6 mm. apart. In hydrogen an initial relatively large drop of total E.M.F. was followed by a rise and finally a decrease,

with the potential difference maintained at a low level. Recovery occurred when pure oxygen was substituted for hydrogen and the conspicuous rebound effect appeared (*cf.* with curves 1, 2, 3, and 8, 9, 10 of figure 3). It is evident that equal and simultaneous change in oxygen concentration changes the localized E.M.F.'s unequally. This would necessarily modify the distribution of E.M.F.'s per unit length which constitute the resultant gradient. The gradient of electric polarity would be different in an atmosphere of air, an atmosphere devoid of oxygen, and an atmosphere of pure oxygen. This conclusion is tantamount to the statement that the oxygen concentration around the root determines to a large degree the characteristic magnitudes of E.M.F.'s per unit length.

In each experiment, when the root-apex exhibited a relatively stable potential difference in (*a*) air, (*b*) hydrogen, and (*c*) oxygen, the distribution of potential difference was determined by the customary method of moving the apical electrode. The position of the breaks (*a*, *b*, and *c*) in curve 4, figure 4, indicates the intervals during which the gradients shown by curves 4a, 4b, and 4c in figure 5 were determined. Curves of the gradients determined in five similar experiments on five different roots are also given in figure 5. Curves 4a, 4b, and 4c show that the gradient was inverted in hydrogen with the apical end electronegative to more basal regions, and that it was displaced to a lower level of potential difference; in oxygen the gradient not only righted itself but also manifested a greater total E.M.F. The dip in each of the curves 4a and 4b, which indicates the existence of components with inverted polarity, disappeared in curve 4c. Gradients corresponding to curves 1a, 1b, 1c, and 6a, 6b, 6c displayed changes practically identical with those exhibited by curves 4a, 4b, and 4c. In hydrogen each gradient was inverted, the apical end became electronegative to relatively more basal regions, and the whole gradient shifted to a lower value; in oxygen each gradient righted itself and curve 6c shows that the corresponding gradient manifested a higher E.M.F. Gradients corresponding to curves 2a and 3a were flattened in hydrogen and became steeper in oxygen with the apical end more highly positive than at any previous period. All the curves in figure 5 obtained by this procedure show that the component parts of each gradient were affected unequally by equal change in oxygen concentration, and that the component E.M.F.'s associated with the tissues in the apical end were displaced to a relatively greater degree than were component E.M.F.'s in the other tissues of the root-apex. An atmosphere of hydrogen tends to make the ends of the gradient more alike; oxygen more unlike. Hydrogen tilts the gradient in a direction opposite to that characteristic of the root-apex in air; oxygen tilts it back to a steeper form. The form of the gradient at any one instant is determined by the concentration of oxygen at that instant.

The curves in figure 5 also throw light on the nature of the phenomena which determine the character of curve 4, figure 4. The initial drop in curve 4 (in hydrogen) is explained by the fact that introduction of hydrogen into the electrode chamber depressed the local polarity potentials in the apical region more than the local polarity of other regions. The high positive potential of the apical end disappeared and the apical end became temporarily electronegative to relatively more basal regions. The rise in the curve in hydrogen is due to the depression of inverted constituent E.M.F.'s and the relatively slow depression of basal components. Finally depression of all the component E.M.F.'s and the relatively greater depression of apical components produced a flattened or inverted gradient and hence a second drop in the curve in hydrogen as shown. In the absence of such definite information as in the foregoing, the initial inverted bayonet-like part of the curve which occurs when oxygen is admitted to the chamber might be explained by either (*a*) a sudden increase in positivity at the basal end, or (*b*) a sudden decrease in the apical end. The results indicate that (*a*) is probably the correct interpretation. Curve 4 shows that the duration of this abrupt change was short. It was followed by a greater increase in positivity in the apical regions than in the basal regions, replacing the gradient to its previous slope in air and manifesting the characteristic rebound. During the rebound the slope assumed its greatest value. At the end of the period in oxygen it was less steep but as a rule the slope was greater than that exhibited by the gradient of the root in air, as the curves in figure 5 show.

The results from this set of experiments, exposing the whole root-apex to change in oxygen concentration, corroborate the conclusions based upon the preceding sets of experiments, in which local regions only were exposed to change in oxygen tension. The effects on the young tissue at the apical end are relatively and absolutely so large in magnitude that they determine the character of the resultant polarity potential of the whole. This illustrates what may properly be called an electrical dominance of the apex.

Absence of significant change in total E.M.F. when oxygen concentration is changed in region outside of electrode contacts

In several of the preceding sets of experiments the hydrogen and oxygen were passed directly into the electrode chamber exposing the whole root and bulb. A sixth set of experiments was therefore carried out in order to determine whether the observed modifications of E.M.F. in hydrogen and oxygen were linked with changes in parts of the root not included in the region between the electrode contacts.

The position of the contacts and jacket is shown in figure 6 (diagrams F and G). The gases were passed through the jacket. Curves 1 and 2 in figure 6

show the results when the chamber was placed as illustrated in the diagrams (F and G). The curves show no significant changes in E.M.F. in hydrogen and oxygen; neither did curves obtained when the periods of exposure were increased to 1-hour intervals. It is evident that the observed changes in E.M.F. concurrent with changes in oxygen concentration were due to changes in E.M.F. in the cells at and between the electrode contacts, and not to changes in neighboring cells. The experiments therefore indicate that the local changes in E.M.F.'s produced by change in oxygen tension are not transmitted in the ordinary sense of conduction of excitation, within the period of the experiments.

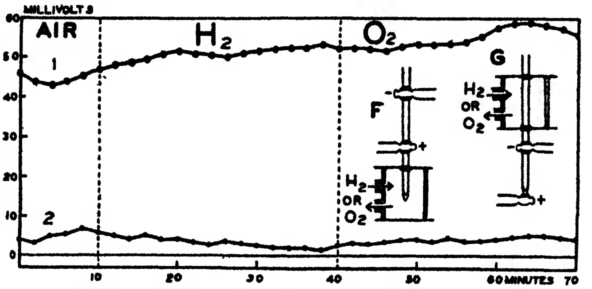

FIG. 6. Absence of effect of change in oxygen concentration when gas jacket is placed outside of electrode circuit. Curve 1 was obtained when gas jacket and electrode contacts were in the position on the root shown in diagram F; curve 2, when gas jacket and electrode contacts were in the positions shown in diagram G.

Discussion

The preceding evidence, together with previously published facts on the respiratory exchange, conclusively establishes the fact of a quantitative linkage between electric polarity and respiration in the root-tip. That region which manifests the greatest magnitude of change in its regional polarity potential is the same region which exhibits the highest positive potential, the largest output of carbon dioxide production and oxygen consumption, the greatest capacity for methylene blue reduction, the highest concentration of sulphydryl groups, and the region in which visible structural differentiation is at a minimum. These facts furnish a complete chain of evidence that morphological, functional, and electrical polarities are interrelated phenomena associated with the oxidative metabolism and with specific differences in the oxidative mechanisms of young and old tissues.

The reversible inhibition of polarity potentials in the root-apex produced by the absence of oxygen is similar to the reversible inhibition of electric polarity in the stem of *Obelia* and in frog skin produced by cyanide, ether, and chloroform (**12, 16**). In the root-apex and in *Obelia* the percentage depression of E.M.F. was greater in the apical region than in the basal region. LUND (**15**) found that the electric polarity of frog skin was

changed by equal changes in oxygen tension on the two sides of the skin. As in the root apex, an oxygen deficit decreased the potential difference and exposure to high oxygen concentration increased the difference. Reversible effects were observed in frog skin but the electric polarity was never completely inverted as in the root-apex.

The characteristic rebound in E.M.F. which occurs when (*a*) the whole "root-apex" or (*b*) the apical end only is exposed to oxygen after a period in its absence, is similar to the rebound effect which has been observed in the E.M.F. of frog skin when the oxygen concentration was changed from a low to a high value (**15**), and the E.M.F. of Douglas fir when the temperature was restored to its original value after a period of exposure to low temperature (**18, 19**). Furthermore, the rebound effect is also similar to the increase in velocity of oxidation, observed by a number of investigators, when certain tissues and organisms were exposed to oxygen after a period in its absence; nerve (**6, 5**), muscle (**5**), tomato fruits (**7**), *Planaria* (**11**), and luminous bacteria (**25, 8**). GUSTAFSON (**7**) also found that, in a given tomato fruit, the magnitude and continuance of increased velocity of oxidation when air was reintroduced into the chamber were dependent upon the length of the period of exposure to pure hydrogen or pure nitrogen. Although he makes no mention of it, his data show that the greatest increase in velocity of oxidation is associated with the younger green fruits as compared with the increase in respiratory activity of older pink or red-ripe fruits. *These rebound phenomena in the velocity of oxidation are obviously to be explained by an accumulation of oxidizable substances during the periods in absence of oxygen or in low oxygen tension, and their increased rate of removal by oxidation upon readmission of oxygen.* In such systems the $\frac{\text{Red}}{\text{Ox}}$ ratios are clearly subject to change by change in the concentration of oxygen. The general occurrence of a rebound effect in the velocity of cell oxidations and the rebound effect in the E.M.F.'s under corresponding conditions of change in oxygen tension leads directly to the conclusion that the mechanism which controls the velocity of cell oxidation also controls the magnitude of the cellular E.M.F.'s.

The gradient of the distribution of cellular E.M.F.'s per unit length, which is an expression of gradation in the rate of output of electric energy quantitatively associated with corresponding differences in velocity of oxidation and structural differences, is distinctly modified by change in oxygen tension. Under the experimental conditions which obtained in this investigation its slope was greatest in oxygen, less in air, and almost obliterated or inverted in hydrogen.

Up to the present the only complete and satisfactory explanation of the experimental results is furnished by LUND's flux equilibrium theory of bioelec-

tric currents and cell oxidation which has been fully developed in a recent paper (**17**). The continuously maintained potentials are the expression of redox states of electromotively active substances in flux equilibrium in polar cells. The observed change in ΣE_p[2] of the root-apex, with equal change in [O] at the apical end only, the basal end only, or the intervening region, is due to unequal changes in $\frac{\text{Red}}{\text{Ox}}$ ratios in the cells of these regions; and accordingly the change in Σ Ep with equal and simultaneous change in [O] at all the specific regions of the root-apex is also due to unequal changes in the $\frac{\text{Red}}{\text{Ox}}$ ratios of the local regions. The experiments show that the greatest change in flux concentrations of electromotively active substances in the cells of a specific region takes place in the young, relatively undifferentiated tissue of the apical end. The change in ΣE_p with equal change in oxygen concentration constitutes the first critical evidence that the velocity of oxidation in young tissue is actually greater than that in old tissue, and that the active mass of oxidizable substance (or system) AH_2 is greater in young tissue. This conclusion is in accordance with recent results (unpublished), obtained in this laboratory by L. M. Henderson, that the percentage increase of oxygen consumption in apical pieces of the root tip (*A. cepa*) was greater than the percentage increase of basal pieces in the same increase in oxygen. It is in agreement with the observations on *Obelia* by Lund (**17**) which showed that the increase in velocity of oxidation in apical halves of the stem was always proportionately greater than the increase in oxidation in basal halves caused by the same increase in oxygen concentration, and that there was a higher concentration of sulphydryl groups in the apical end. Finally, the conclusion that the effective concentration of oxidizable substance (or system) AH_2 is proportionately

[2] ΣE_p expresses the polarity potential of a polar tissue or organ. It is the algebraic sum of the polarity potentials (E_p) of the individual cells.

A simplified form of the electrochemical equation which expresses the polarity potential E_p of a single cell as given by Lund is:

$$E_p = \frac{RT}{2F} \ln \frac{[A]_b \quad [AH_2]_a \quad [O]_a}{[AH_2]_b \quad [A]_a \quad [O]_b}$$

where R is the gas constant; T the absolute temperature; ln the natural logarithm; F, the Faraday equivalent; and $[A]_a$, $[A]_b$, $[AH_2]_a$, $[AH_2]_b$, $[O]_a$, $[O]_b$ the concentrations of electromotively active substances. For the polarity potential ΣE_p of a polar tissue the equation may be expanded to

$$\Sigma E_p = \left[\frac{RT}{2F} \ln \frac{[A]_b \quad [AH_2]_a \quad [O]_a}{[AH_2]_b \quad [A]_a \quad [O]_b}\right]_1$$

$$\pm \left[\frac{RT}{2F} \ln \frac{[A]_b \quad [AH_2]_a \quad [O]_a}{[AH_2]_b \quad [A]_a \quad [O]_b}\right]_2 \pm \left[\ldots\ldots\right]_3 \pm \left[\ldots\ldots\right]_n$$

where the subscripts 1, 2, 3, etc., denote different cells.

much greater in the apical end is supported by the recent observations mentioned above that the concentration of sulphydryl substances is distinctly more concentrated in the cells just behind the root cap. The fact that, as a rule, after an interval of 20 or 30 minutes in oxygen, the slope of the gradient which expresses the ΣE_p per unit length is greater than its slope in the initial period in air, shows that part of the surplus of oxidizable substances accumulated during the period in hydrogen is still available or that an increase in the velocity of the reaction $X \rightarrow AH_2$ occurred and more AH_2 is continuously available. Concomitant with the difference in magnitude of rebound in E.M.F. in apical and basal ends of the root-apex is the apparent unequal acceleration of the reaction $AH_2 + O \rightarrow A + H_2O$ in apical and basal ends. Both the concentration of oxygen and the concentration of AH_2 are limiting factors which determine the magnitude and orientation of electric polarity in the root-apex. The conspicuously greater accumulation of electromotively active material in the apical end indicates that the mechanism of accumulation of such a large surplus of oxidizable substances is a definite characteristic of young tissue.

Oxidation-reduction potentials are not, of course, the only means by which differences of potential are, or may be, established in living cells, and the flux equilibrium is not the only type of electrochemical equilibrium which may exist in the cell; but it is the only type thus far considered in the literature of electrophysiology which can maintain in a direct manner a continuous output of electric energy. The theoretical requirements of the electrochemical equations which express the relation of the observed maintained E.M.F.'s to the velocity of cell oxidation are given by LUND (**17**). The facts derived from the present investigation on the root tip, which is a polar tissue, specifically satisfy these requirements and therefore furnish direct evidence of the validity of the flux equilibrium concept.

The experimental results appear to be of vital significance for cell dynamics and structural differentiation. Very direct evidence for this has been found in experiments on the effect of applied electric currents, now in progress. The polar tissues of the root are constantly generating electric energy, and the flux equilibrium is a state maintained, at least in part, by the relations between the intake of oxygen and the concentration of available substances for oxidation in the individual cells. The structural differentiation of the cells and tissues has associated with it an electrical differentiation. The facts show that this is primarily a difference which depends for its significance upon the *intensity* factor of cell oxidation as expressed by oxidation-reduction potentials at oriented loci. There is an orientation of energy output in cells of polar tissues, and the observed E.M.F. expresses the algebraic summation of local forces. In low oxygen concentration the observed output of electric energy is diminished, in high

oxygen concentration it is increased. Continuously maintained oxidation-reduction potentials are an expression of metabolic activities associated with output of electric energy, and from this it may be concluded that oriented *electrometabolism* is characteristic of polar tissues.

It is well known that oxygen is necessary for the proper functioning of roots. Its absence inhibits growth, modifies cell division, and affects geotropic curvature.[3] It has been shown that the amount of available oxygen is also related to the rate of absorption of water and solutes by roots (**10**, **23**, **24**, **9**). It is highly probable that the energy required for one or more of these oriented processes is derived to greater or lesser extent from the oriented continuous bioelectric currents in the root.

Summary

1. Evidence is presented which shows that the continuously maintained E.M.F.'s in the onion root (*Allium cepa*) are quantitatively linked with oxidative metabolism. By means of new apparatus and technique, described in the text, it was possible to measure the effect on the E.M.F. of the uninjured intact root when oxygen or other gases were applied to a root region of 1 mm. or more in length without mechanical stimulation and change in humidity or temperature. The investigation involved experiments on the distal 6 ± mm. of the root, a region designated as the "root-apex" for convenience.

2. Change in oxygen concentration at (*a*) the apical region (1.5 mm.), (*b*) the basal region (1.5 mm.), and (*c*) an intermediate region of the root-apex, respectively, changes the regional polarity potential of each region and correspondingly modifies the total E.M.F. of the root-apex in each case.

3. Simultaneous equal change in oxygen concentration at all the regions of the root-apex has an unequal effect on E.M.F. of the different regions of the root-apex. This is due to the fact that the effect in the young, relatively undifferentiated tissue of the apical end is relatively and absolutely so large in magnitude that it determines the character of the resultant polarity potential of the whole.

4. The effect of change in E.M.F. produced by change in oxygen concentration is reversible. In hydrogen the E.M.F. of a given region is diminished; in oxygen the E.M.F. is increased.

5. The slope of the gradient which represents the output of electric energy per unit length of the root-apex is different in (*a*) air, (*b*) hydrogen, and (*c*) oxygen. The gradient is flattened or may be inverted in hydrogen. Its slope is steepest in oxygen.

[3] A review of the literature previous to 1921 is given by CLEMENTS (3). Recent contributors are BOUYGUES (1), NAVEZ (21), NAVEZ and CROZIER (22), and ZIMMERMAN (26).

6. The observed changes in E.M.F. concurrent with changes in oxygen concentration were produced by changes in E.M.F. between and at the electrode contacts and not by changes outside of the electrode circuit. These facts constitute additional confirmation of the validity of the principle of algebraic summation of individual cell E.M.F.'s.

7. The results show that the velocity of oxidation is greatest in the cells actively engaged in cell multiplication.

8. The typical rebound phenomenon which is apparently produced by the accumulation of oxidizable substances in the absence of oxygen is greatest in the young (apical end) tissue.

9. The results furnish additional direct evidence for the validity of the theory that continuously maintained E.M.F.'s are generated by the redox system of the cell. The magnitude of the E.M.F. at any instant depends upon the conditions of a flux equilibrium in the process of cell oxidation.

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PHYSIOLOGY OF APPLE VARIETIES[1]

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(WITH SIX FIGURES)

Introduction

A better understanding of physiological factors determining the growth and fruiting habits of different apple varieties (*Malus malus*) should suggest answers to many common orchard problems. Why, for example, do trees of some varieties habitually bear biennially while trees of others bear regularly, although given the same cultural treatment? Why do trees of some varieties come into profitable fruiting five to six years after planting while others do not fruit commercially until ten to twelve years of age? Why does one variety bear regularly for one grower and biennially for his neighbor? Other orchard conditions present similar problems for practical solution.

The work described in this paper is the result of studies conducted at the University of Wisconsin during 1927, 1928, and 1929 in an effort to find physiological causes for the differences in growth and fruiting of some varieties of apples.

PREVIOUS DATA SUGGESTING AN APPROACH TO THE PROBLEM

A direct relation between growth and fruiting character has been suggested for trees of the Wealthy variety (5). Differences of growth character and fruitfulness were produced by varying the environmental conditions under which the trees were grown. Strongly vegetative trees making a long, slender growth with a high nitrogen nutrient and also weakly vegetative trees making a short, slender growth with a low nitrogen nutrient were non-fruitful. Between these two extremes of vegetative condition were trees having a relatively thick and moderately long growth which was fruitful.

Chemical analyses of the different types of growth revealed that the unfruitful strongly vegetative trees were high in nitrogen and low in carbohydrates, especially starch; that the weakly vegetative unfruitful trees were low in nitrogen and high in carbohydrates; and that the fruitful trees were of intermediate composition. Axillary and terminal buds were common on trees that were fruitful but which approached the strongly vegetative condition, while fruit bud formation was largely limited to spurs on trees that

[1] Publication authorized by the Director of the Wisconsin Agricultural Experiment Station.

approached the weakly vegetative condition. Between these two conditions fruit buds were produced in abundance laterally, terminally, and on spurs.

The cause of the variation in growth and fruiting within a variety has been thought (**2, 5, 6**) to lie in a difference in chemical composition, particularly as regards the relation of carbohydrates and nitrogen compounds. If this hypothesis is correct, it was reasoned that a difference in the formation and utilization of carbohydrates and compounds of nitrogen might account to a degree for the difference in growth and fruiting habits of apple varieties.

Materials

For convenience in studying the factors underlying their variable fruiting behavior, apple varieties were classified according to their usual bearing habits, as follows:

1. Biennially bearing varieties, in which a heavy crop tends to alternate each year with little or no crop under a rather wide range of orchard conditions.
2. Regularly bearing varieties, which tend to bear a satisfactory commercial crop each year when given relatively good cultural conditions.
3. Shyly bearing varieties, which tend to come into bearing late and which, for several years after beginning to bear, usually produce a light crop either biennially or regularly.

It is not presumed that such an arbitrary classification holds for all varieties under all conditions. Trees of any variety may be found in any one of the classes, provided the proper environmental conditions obtain. Trees of all varieties tend to come into the first class as they grow older. Cultural and climatic conditions influence markedly the class into which a variety falls.

Certain varieties were studied as representative of each class. Duchess and Wealthy were taken as typical of the biennially bearing varieties; Fameuse, Northwestern, and McIntosh of regularly bearing varieties; Spy and in some cases Liveland or Newell of shyly bearing varieties.

Rate and period of terminal growth.—The amount and period of terminal growth were obtained from trees growing in commercial orchards at Sturgeon Bay, Wisconsin. The growth curves for the biennially bearing Duchess and Wealthy, the regularly bearing McIntosh and Northwestern, and the shyly bearing Spy and Liveland were obtained from trees growing in the same orchard under similar soil and cultural conditions. Data upon the regularly bearing Wealthy and Duchess were obtained from rather heavily pruned and nitrated trees in a second orchard. Data for biennially bearing McIntosh, Fameuse, and Northwestern were obtained from trees in a third well cultivated but poorly fertilized orchard on light soil. The

biennially bearing Spy and Newell were old, lightly pruned trees growing in a fourth orchard receiving some cultivation but no fertilizer.

Xylem formation and starch disappearance and accumulation.—Xylem formation and starch disappearance or accumulation were observed in the current season's growth or in one- and two-year old wood of some of the trees just described.

Macrochemical analyses.—Samples for macrochemical analyses consisted of the entire current season's terminal shoots from trees growing in commercial orchards at Sturgeon Bay, as previously described. Such material does not represent the highest or lowest extreme of carbohydrate or nitrogen content, but approximates that accompanying extremes of fruiting condition.

Anatomical structure.—This was studied in terminal shoots from trees of commercial orchards at Sturgeon Bay, in nursery trees the first season after being grafted and growing under uniform soil conditions in a nursery at Madison, and in yearling trees propagated on dwarf stocks growing in pot culture with controlled nutrients.

Methods

Sampling.—The method of sampling employed was carefully to select typical samples of the class or group being observed, rather than to sample at random or in large numbers and draw conclusions after mathematical treatment of the data. In all cases where terminal shoots were used, they were selected for a particular character or quality of growth (fig. 1), as these have been observed to be associated with fruiting performance of the variety being sampled. The character of the terminal growth differs with variety as regards length, diameter, tapering, bark color, willowyness, brashness, length of internodes, and size of terminal leaves. In sampling the nursery trees and those growing in pots, care was taken to obtain samples typical of the variety or plot, selections being made on the basis of growth character.

Measurement of terminal growth.—Records of the rate of growth of the terminal shoots were obtained by measuring the length of the terminal shoot about every five days during the growing season. Data were taken in 1927 and 1928 but only the 1928 results are presented, as they are typical of the two seasons.

Xylem formation and starch disappearance and accumulation.—Samples from terminal shoots and one- and two-year old wood were collected at about five-day intervals during the spring of 1927 and 1928 at Madison and during the spring and summer of the same years at Sturgeon Bay. These were stored in formalin-alcohol-acetic acid solution (100 cc. of 50 per cent. alcohol, 2–3 cc. of acetic acid, and 7 cc. of 40 per cent. forma-

FIG. 1. Typical shoots of various fruiting classes.

A. Fruitful terminal shoots of biennially bearing trees. Left, Duchess; right, Wealthy. These shoots tend to have large terminal leaves arising at wide angles, long terminal internodes, and a uniformly thick diameter.

B. Terminal shoots of regularly bearing trees. Left, Fameuse; right, McIntosh. These shoots tend to have medium sized terminal leaves, moderately long terminal internodes, and a medium diameter which tapers somewhat.

C. Terminal shoots of shyly bearing trees. Left, Spy; right, Newell. These shoots tend to have rather short terminal internodes, small terminal leaves arising at a sharp angle, and a small tapering diameter.

D. Unfruitful terminal shoots of biennially bearing trees. Left, Duchess; right, Wealthy. These shoots tend to have short terminal internodes, small terminal leaves arising at sharp angles, and a slender diameter.

lin). Freehand transverse sections were subsequently made: (1) at a point usually in the third internode from the tip; (2) at the middle of the shoot; and (3) at the base of the shoot. For the studies involving two-year old wood similar sections were made at corresponding points. The sections

were stained with a solution of iodine in potassium iodide and mounted in a glycerin solution of the same to render the reaction somewhat permanent. The width of secondary xylem was measured in units of an eyepiece micrometer and recorded together with the estimated amount of starch. Starch storage may occur throughout the pith parenchyma but is usually more pronounced in the outer areas. It was observed in xylem and ray parenchyma and in phloem and cortical parenchyma. The amount of starch present was estimated from a consideration of the relative proportion of parenchyma cells of each tissue containing it, and also from the relative abundance with which it occurred within such cells.

Macrochemical analyses.—Material for chemical analyses was collected on August 12, 1927, and July 27, 1928, at Sturgeon Bay, Wisconsin. On these dates, fruitfulness of the shoots might be expected to be related to their composition, if such relation exists, for blossom primordia were first observed during the early part of August each year. Data obtained in 1927 were again in agreement with those of 1928 and are not shown. The material was dried at 100° C. for a period of about one-half hour, followed at 60° C. until an approximately constant weight was reached. Analyses were made the following winter in each case. The dried samples were ground to pass a no. 80 mesh screen. The unmodified Kjeldahl method was used for determination of nitrogen, as little nitrate nitrogen has been reported in apple wood. Official methods of the Association of Official Agricultural Chemists were used in making carbohydrate determinations.

Anatomical structure.—Transverse sections 15–30 μ thick were made with the sliding microtome from samples fixed and stored as previously described. These were stained with safranin and aniline blue or with safranin and Delafield's haematoxylin and mounted in balsam. Camera lucida diagrams of cross sections of pith tissue were made from material prepared in this manner.

High and low nitrogen nutrient.—Yearling trees of several varieties propagated on dwarf stocks were grown in the greenhouse in 14-inch pots containing practically pure quartz sand from January, 1928, to May, 1928, and then transferred to out-of-doors for the remainder of the season. Stock nutrient solutions prepared according to the following formula were used:

With nitrogen

A

KNO_3	40 gm.
$MgSO_4$	20 "
$Ca(H_2PO_4)_2$	20 "
Water	920 cc.

B

$Ca(NO_3)_2$	60 gm.
Water	940 cc.

Without nitrogen

A

$MgSO_4$	20 gm.
K_2HPO_4	20 "
KH_2PO_4	10 "
Water	950 cc.

B

$CaSO_4$	Saturate
Water	1000 cc.

The nutrient solution as supplied to the potted trees was prepared by diluting A and B each with seven parts of water and mixing together. One-half liter of this nutrient was added to each pot at about 10-day intervals during the period of most active growth, and less often later in the season. Six to eight trees of each variety were given the nutrient containing nitrogen and an equal number given the nutrient without nitrogen. All trees were watered with tap water which contained traces of nitrogen. The records describing the growth made by the trees during 1928 (table III) were taken in October. The trees were then removed from the pots and stored in the cellar until January, 1929, when they were again potted and placed in the greenhouse but no nutrient other than that contained in the tap water was supplied.

Definitions

The terms fruitful shoot and fruitful growth have reference to the growth produced when fruit buds are formed and not when fruit is actually borne. (The blossoms appearing on a tree in May were differentiated the previous summer.) The terms non-fruitful shoot and unfruitful growth refer to the growth produced when no fruit buds are formed. Thus, on biennially bearing trees, a fruitful growth is made the season when the tree is not bearing fruit, and an unfruitful growth when the tree is bearing fruit.

In the discussion of the anatomical structure of the xylem, the terms spring wood and summer wood are used. These terms designate a quality of the xylem rather than a time of formation. The first formed inner xylem, in which vessels are large and abundant, is spoken of as spring wood although not always formed in the spring. The last formed xylem, in which vessels are few and small and in which parenchymatous and fibrous cells are abundant, is spoken of as summer wood or summer xylem.

Presentation of data

Character of terminal shoot growth.—The quality of the terminal shoots as to length, diameter, degree of tapering, length of internodes, brashness, and size of terminal leaves is thought to be indicative of the physiological condition of the tree.

The character of the terminal shoot growth produced by trees of representative varieties and fruiting types is shown in figure 1. The fruitful terminal growths made by trees of biennially bearing varieties were found to taper but little from base to tip, terminal internodes were long, and the last leaves formed were large, the wood itself being brashy. The unfruitful terminal shoot growth made by trees of biennially bearing varieties was willowy and tapered markedly from base to tip, terminal internodes were short, and last formed leaves were small. The terminal shoot growth made

by trees of regularly bearing varieties was observed to be moderately brashy and moderately thick from base to tip, terminal internodes were moderately long, and last leaves formed were moderately large. The terminal shoot growth made by trees of shyly bearing varieties was somewhat willowy and slender, terminal internodes tended to be short, and last formed leaves moderately small.

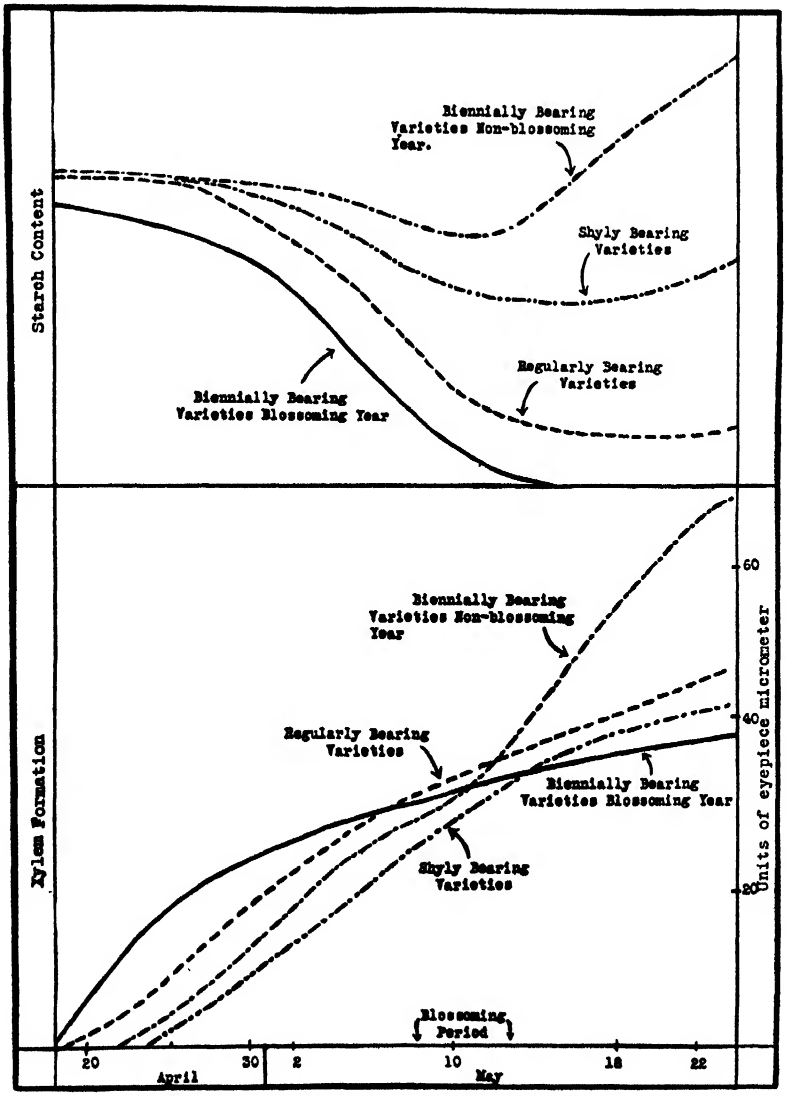

FIG. 2. Formation (width) of new xylem and relative starch content near tips of previous season's growth. Estimated from microscopic observations, spring of 1928, Madison, Wisconsin.

This description of growth character applies only when trees are fruiting typically for the variety. When a tree of a variety of one class fruited like trees of another class of varieties, the character of the terminal shoots produced was found to be correlated to and typical of the condition of fruitfulness.

STARCH DISAPPEARANCE.—The rate and completeness with which starch and cell wall thickenings (reserves) disappeared from the previous season's shoots prior to, during, and following blossoming were studied as an indication of the relative carbohydrate utilization in the shoots of the different classes and varieties of trees.

The disappearance of starch from the previous season's shoots was first initiated near growing points, as apical meristems and blossom buds, in biennially bearing trees which were entering the blossoming year (fig. 2). This disappearance was followed in point of time by regularly bearing trees, shyly bearing trees, and biennially bearing trees which were entering the non-blossoming year.

In the blossoming year of the biennially bearing trees starch disappearance continued until after blossoming, when little remained in any of the tissues of the small branches. A large part of the reserve present as cell wall thickenings in the pith also disappeared, particularly in regions close to blossom buds, apparently being utilized in fruiting and in growth.

In the non-blossoming year of the biennially bearing trees, starch disappeared but little from the phloem, cortex, and xylem; a considerable amount remained in the pith. Starch which disappeared with the starting of growth in the spring was soon replaced and the content remained relatively high throughout the summer. Few if any of the cell wall thickenings of the pith disappeared.

In the regularly bearing and in the shyly bearing trees starch was depleted less rapidly and less completely than in the blossoming and fruiting year of biennially bearing trees, but more rapidly and more completely than in the non-fruiting year of biennially bearing trees.

RATE AND PERIOD OF TERMINAL GROWTH.—As has been suggested by many workers, the rate and period of terminal growth of a shoot may be influenced by the composition or nutritional condition of the shoot; hence shoots differing with respect to nutrition should have a different rate and period of terminal growth. With this thought in mind, the rate and period of elongation of the terminal shoots of trees of different varieties and classes of growth and fruiting were measured. The data for 1928 only are shown (fig. 3), being alike for the two years observed. All shoots of the class of which each curve is representative had growth curves markedly uniform as to configuration, although the total length of the shoots measured was found to vary within a class.

The rate and period of terminal growth of the unfruitful terminal shoots of biennially bearing trees were found to be markedly different from that of the very fruitful growth of biennially bearing trees. The unfruitful shoot started growing later, its growth proceeded slowly during the early part of the growth period, it put on smaller leaves, and ceased elongation abruptly. The fruitful growth started much earlier and more rapidly, put on large leaves, and ceased elongation gradually. The growth of the

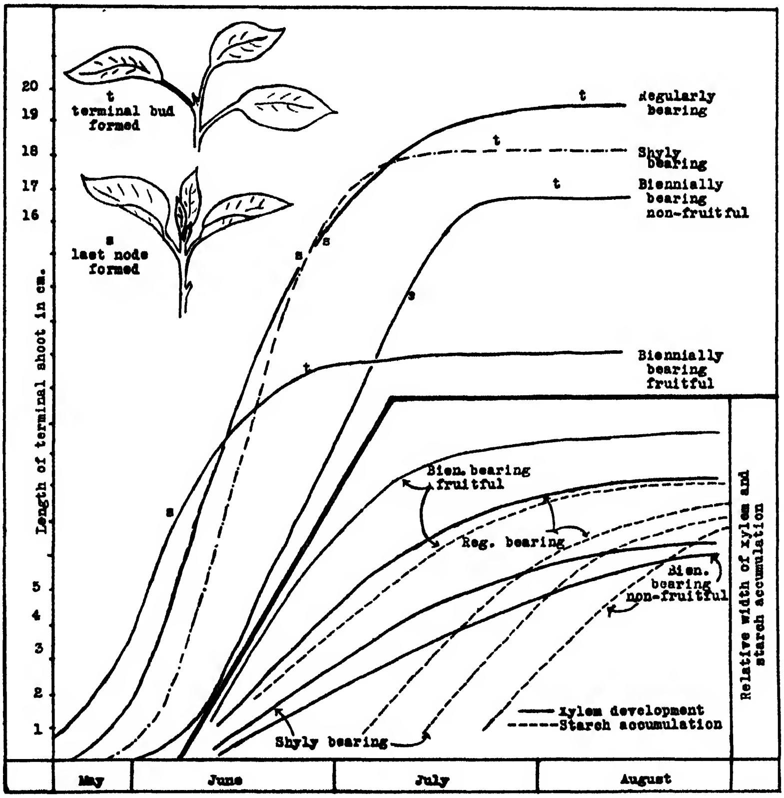

FIG. 3. Curves of terminal shoot growth, xylem development, and estimated starch accumulation in terminal shoots (third internode from tip) of trees of the various growth and fruiting classes. Sturgeon Bay, 1928.

terminal shoots of the regularly bearing trees was intermediate in respect to time of starting growth, initial rate, and abruptness with which growth ceased. This was particularly true when equal lengths of all types of shoots were compared. Long terminal shoots of regularly bearing trees started growing in the spring nearly as fast as shorter but more fruitful growths of biennially bearing trees. The leveling of the growth curve of the terminal shoots of the shyly bearing varieties (Spy and Liveland) suggested

that of the curves for the unfruitful growths of biennially bearing trees.

The relative amount of terminal elongation of the shoots after the last node has been formed (fig. 3, *S*) is of interest and possibly of significance. This elongation is due to lengthening of internodes. It may be that the short terminal internodes of unfruitful shoots and the abrupt flattening of their growth curve are related to a common fundamental condition, and likewise the long terminal internodes and gradual flattening of the growth curve of the fruitful shoots.

If the form of the growth curve is influenced by the internal nutrition or composition of the shoot, then one interpretation that may be made of the elongation curves of figure 3 is that terminal shoots of trees of the different varieties and classes are unlike as to composition. The curves of the fruitful and of the unfruitful terminal shoots of biennially bearing trees possibly represent extremes of composition, those of shoots of regularly and shyly bearing trees indicating an intermediate composition.

Xylem formation.—A correlation of diameter thickness to fruiting performance has been suggested for spurs by Crow and Eidt (1) and for spurs and terminal shoots by Roberts (5).

The formation of secondary xylem in the current season's terminal shoots of trees of the different varieties and classes was found to be related to fruitfulness; that is, to blossom bud formation, in the shoots (fig. 3). It was initiated first, and proceeded most rapidly in the fruitful terminal shoots of biennially bearing trees. It was next initiated and proceeded moderately rapidly in the terminal shoots of regularly bearing trees. It was initiated later and proceeded slowly in the terminal shoots of shyly bearing trees. It was initiated last and proceeded most slowly in the unfruitful terminal shoots of biennially bearing trees.

The widest xylem (measured at a point usually in the third internode below the tip of the shoot) was in the fruitful terminal shoots of biennially bearing trees. The terminal shoots of regularly and shyly bearing trees had the next widest xylem, and the unfruitful terminal shoots of biennially bearing trees had the narrowest xylem (figs. 4, 5). The xylem at the base of the shoot may be wider in a long growth such as a sucker or the terminal shoot of a regularly bearing tree than in the fruitful shoot. The width of the xylem in these cases, however, appears to be associated with the elongating of the shoot. The quality of such basal xylem, as indicated by anatomical structure, was not like that in the fruitful shoot. Spring xylem development in one-year old wood follows the initiation of starch disappearance and the initiation of terminal shoot growth. It is correlated with these and not directly with blossom bud formation.

Starch accumulation.—Starch accumulation in the terminal shoots was investigated as a possible index of the carbohydrate composition of the

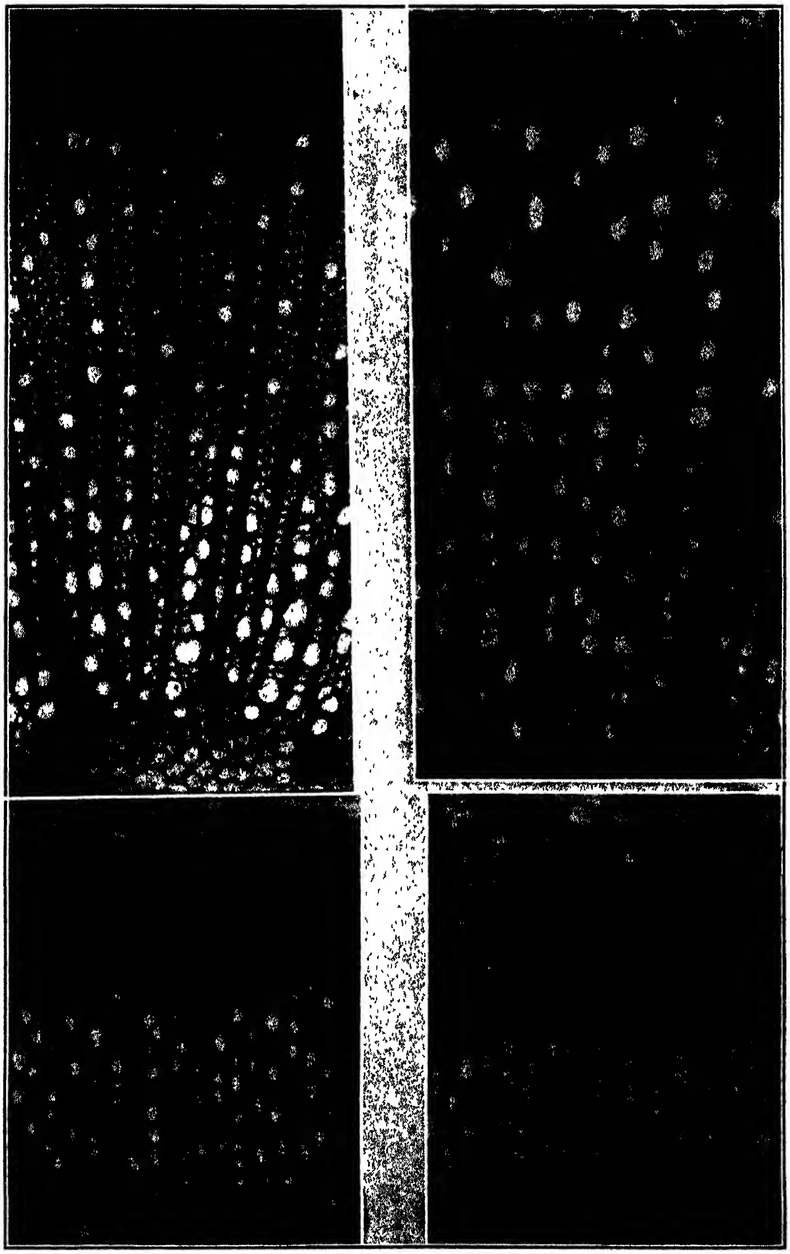

Fig. 4. The xylem in the fruitful growths of biennially bearing trees is much wider than that in the unfruitful growths. It has an abundance of summer wood containing many parenchymatous cells.

A. Wealthy fruitful. B. Duchess fruitful.
C. Wealthy unfruitful. D. Duchess unfruitful.

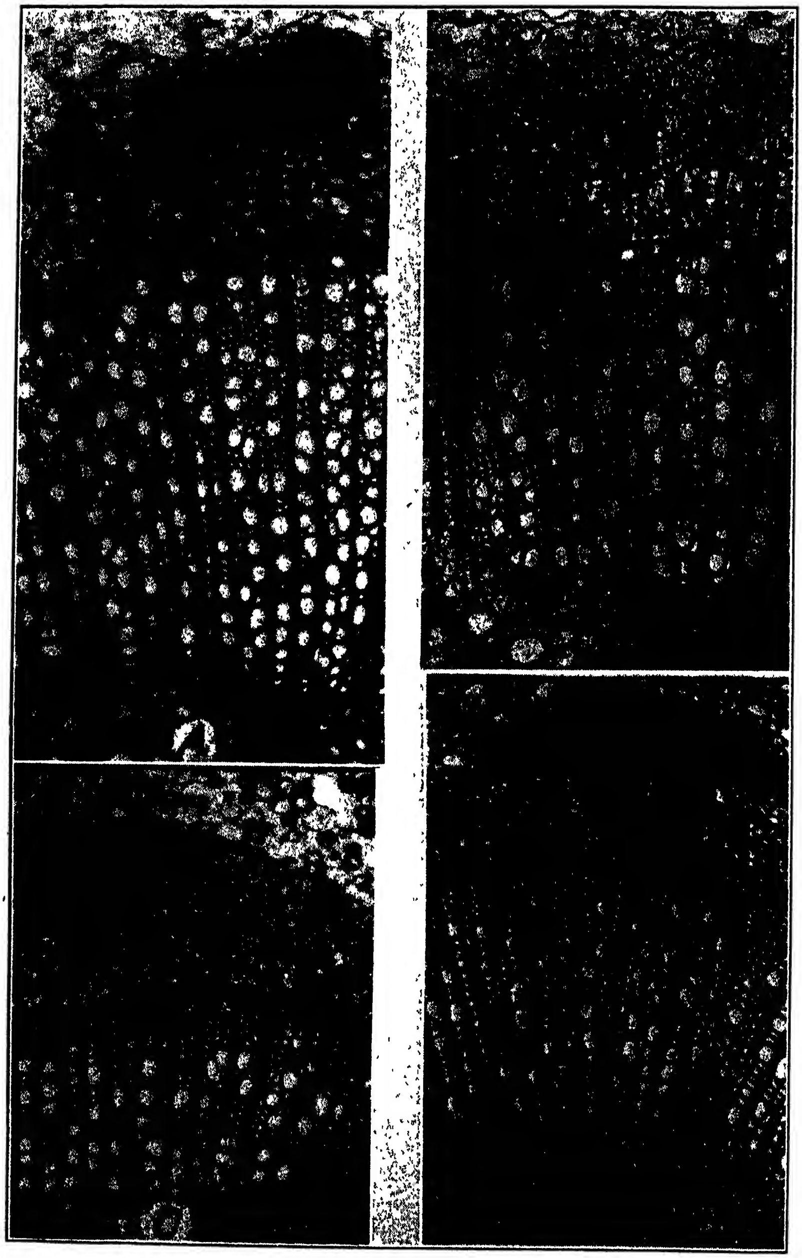

FIG. 5. The xylem in the terminal shoots of regularly and shyly bearing trees is intermediate in width and amount of summer wood but that of the shyly bearing approaches the non-fruitful extreme.

A. Northwestern. B. McIntosh.
C. Newell. D. Spy.

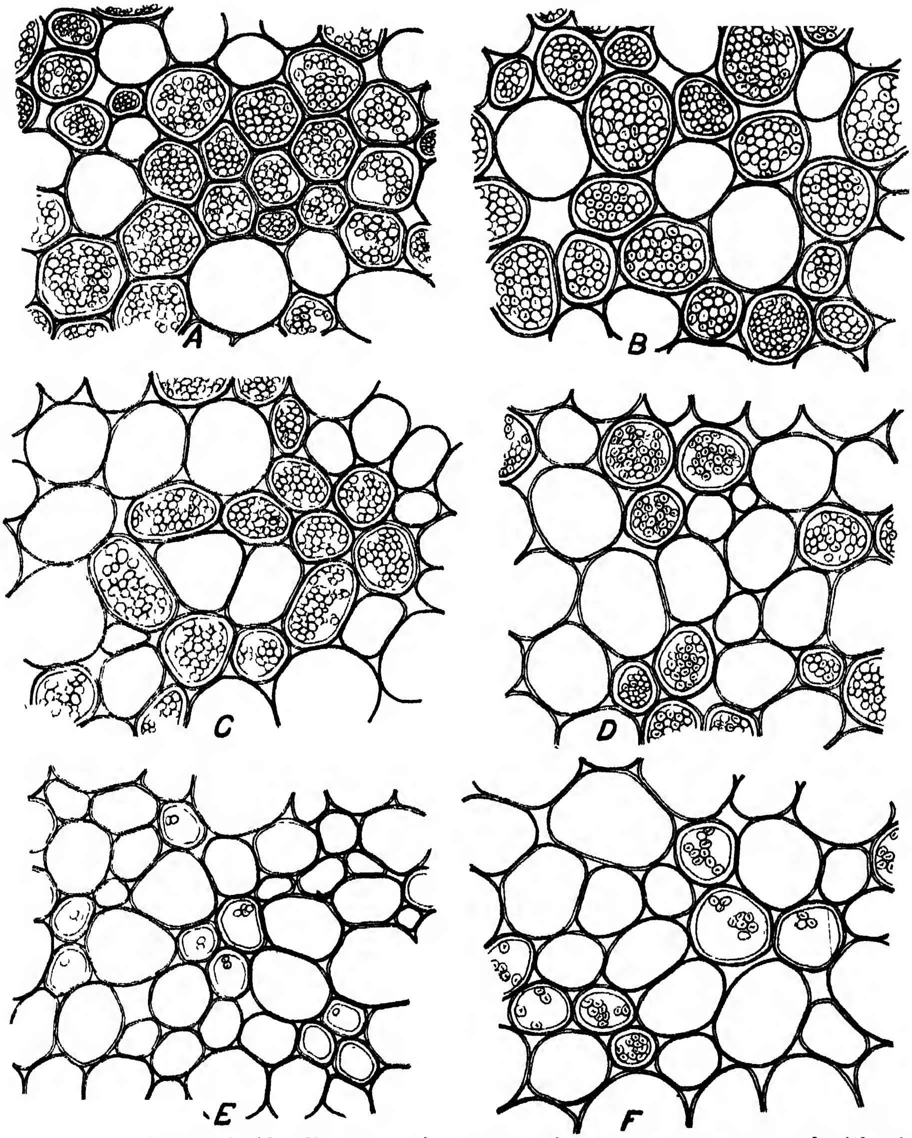

FIG. 6. Camera lucida diagrams of representative areas near center of pith at base of nursery whips.

A. Northwestern girdled. B. Wealthy untreated.
C. Northwestern untreated. D. Fameuse untreated.
E. Spy untreated. F. Northwestern defoliated.

different shoots and as one factor associated with their fruiting performance. In the current season's terminal shoots, starch accumulation begins at the base and proceeds upward. This accumulation and the formation of the

xylem progress uniformly in the current season's growth. In older wood the presence of lateral branches and spurs causes local accumulations of starch and irregularities in width of xylem.

Starch appeared first in the storage tissues of the fruitful terminal shoots of biennially bearing trees, and last in the unfruitful shoots of biennially bearing trees. The appearance of starch in the terminal shoots of typically bearing trees of regularly and shyly bearing varieties occurred after that in the fruitful shoots but preceded that in the unfruitful shoots of the biennially bearing trees (fig. 3).

The formation of the secondary thickenings of the pith cell walls appeared to precede the formation of starch in the cell. Starch was not found in pith cells which did not have secondary thickenings (fig. 6). Inversely it was observed that starch is removed from pith cells in the spring before the wall thickenings disappear. The total width of the thickenings was observed to vary between the different types of shoots, as does also the relative number of cells in the pith having such wall thickenings.

A considerable amount of starch was present in the fruitful growths by June 20 (1928), whereas a comparable amount had not accumulated in unfruitful terminal shoots until the middle of July. About the first of August starch began to accumulate abundantly in all shoots. By the early part of September, it appeared that nearly as much starch was present in the unfruitful as in the fruitful growths, yet blossom buds were formed only on the fruitful shoots where starch storage took place early in the season. By the latter part of September or early October starch began disappearing from the terminal shoots. This disappearance was observed to be initiated at the base of the shoot and proceed upward.

Chemical composition of terminal shoots.—Data obtained from macrochemical analyses of tissue collected in 1928 appear in table I. The values found for percentage of ether extractable materials, free reducing and total reducing substances, do not show a consistent relation to growth character or to the amount of blossom bud formation in the shoot. The acid hydrolyzable fraction was somewhat correlated with the degree of fruitfulness of the shoots of different varieties. Of the samples analyzed, starch content was found to be highest in the fruitful shoots of biennially bearing trees and lowest in the unfruitful shoots. The starch-nitrogen and total carbohydrate-nitrogen ratios were greatest in the fruitful and lowest in the unfruitful shoots. Evidently typically fruiting trees of biennially bearing varieties build up carbohydrates abundantly in relation to nitrogenous compounds the season that many fruit buds are formed. The next season, when the fruit is borne and no blossom buds are formed, they are relatively low in carbohydrates. Trees of regularly and shyly bearing varieties formed an intermediate number of fruit buds, and produced a terminal

TABLE I

COMPOSITION OF TERMINAL SHOOTS COLLECTED JULY 27, 1928, STURGEON BAY, WISCONSIN

	Duchess unfruitful	Wealthy unfruitful	Northern Spy	North-western Greening	McIntosh	Duchess fruitful	Wealthy fruitful
Percentage ether extract	2.98	3.32	2.38	2.41	2.20	2.38	2.36
Percentage free reducing substances	2.96	3.07	2.18	2.67	2.31	2.57	2.59
Percentage total reducing substances	4.87	5.45	3.14	5.23	4.50	4.62	4.33
Percentage starch	2.41	2.57	3.91	2.86	2.95	4.86	5.95
Percentage acid hydrolyzable substances	5.03	5.41	5.27	5.54	6.77	6.27	6.09
Percentage total carbohydrates	12.31	13.43	12.32	13.63	14.22	15.75	16.37
Percentage total nitrogen	0.930	0.868	0.759	0.830	0.894	0.792	0.777
Ratio $\frac{\text{starch}}{\text{nitrogen}}$	2.59	2.80	5.15	3.44	3.30	6.13	7.65
Ratio $\frac{\text{total carbohydrates}}{\text{nitrogen}}$	13.20	15.5	16.20	16.40	16.00	19.80	21.1

growth that was intermediate in carbohydrate and nitrogen composition between that produced during the "on" and "off" years of biennial kinds.

Shoots selected at random from those collected for macro-analyses were sectioned and observed microscopically. The results of these studies were in general agreement with the analytical data, although the former indicate wider extremes of starch and hemicellulose content than were revealed in the macro-analyses.

These data cannot be interpreted as meaning that a maximum carbohydrate content makes for fruitfulness, since samples were taken only from the "fruitful range." An unfruitful high carbohydrate-low nitrogen sample (**3**) was not included.

The numerical values assigned to the ratios in table I are relative and not absolute. An absolute expression of chemical composition does not seem feasible. This is partly because of the failure of present methods of macro-analyses accurately to measure materials used in growth and fruiting. As in the present data, the ordinary methods of analyses recover only a part of the total material in the sample, any metabolic value that the remainder may have not being estimated. Again there is the question of whether data showing composition should be expressed as percentage or in amounts per given unit. Anatomical studies have indicated that fruiting is associated with a quality of composition rather than with a quantity of materials. If such is true the percentage basis may provide a more accurate index by which the composition as related to fruiting may be expressed.

Anatomical structure.—That the anatomy of the plant may reflect its vegetative and fruiting condition has been suggested by numerous workers, yet little use has been made of this method as one approach to the solution of certain physiological problems. In the present study consideration of anatomical features provided additional information.

The xylem was found to vary widely in the different classes of shoots as to amount and quality (numbers and kinds of cells, size of cells, etc.) of summer wood present. The xylem of the fruitful growths of biennially bearing trees had the most summer wood; that of the unfruitful terminal growths had the least (fig. 4). Intermediate between these two extremes was the amount of summer wood present in the xylem of the terminal shoots of regularly and shyly bearing trees (fig. 5).

The summer wood characteristic of the fruitful terminal shoot of a biennially bearing tree was found to have fewer vessels than the spring wood of the same shoot, or of the spring wood comprising practically the entire xylem in the unfruitful shoot. Other differences were also found which may have much significance from the standpoint of the chemical composition of the shoot, and particularly of changes in composition throughout the period of xylem formation. Outstanding among these were

the numbers and ratios of ray cells, vessels, fibers, and parenchyma (table II and figs. 4 and 5).

TABLE II

RATIOS OF FIBERS, RAY CELLS, AND VESSELS TO PARENCHYMA IN OUTER XYLEM OF SHOOTS OF VARIOUS VARIETIES GROWING UNDER SEVERAL CONDITIONS

	FIBERS: PAREN-CHYMA	RAY CELLS: PAREN-CHYMA	VESSELS: PAREN-CHYMA
TERMINAL SHOOTS OF ORCHARD TREES			
Wealthy unfruitful	5.8	......	... ...
Duchess unfruitful	5.9	....	....
Spy	5.5	...	.. ...
Newell	5.4	...	...
Northwestern	5.2	1.8	0.74
McIntosh	4.6	1.7	0.75
Fameuse	3.3	1.3	0.40
Wealthy fruitful	2.4	0.72	0.19
Duchess fruitful	2.5	0.74	0.20
Wealthy, under-vegetative and unfruitful	2.0	0.80	0.20
BASE OF NURSERY WHIPS			
Spy	11.6	1.7	0.58
Fameuse	6.0	0.86	0.44
Wealthy	4.6	0.65	0.36
Northwestern, defoliated	12.9	1.8	0.65
Northwestern, untreated	6.2	0.98	0.30
Northwestern, girdled	3.8	0.76	0.35
POTTED WEALTHY TREES			
Grown in shade with high nitrogen nutrient, girdled, sample taken below girdle	13.3	7.4	1.9
Grown in full sunlight with low nitrogen nutrient, girdled, sample from above girdle	2.5	1.4	0.45

The summer xylem of the fruitful terminal shoots of biennially bearing trees had a relatively large number of parenchymatous cells, and the fiber to parenchyma ratio was low. The summer xylem of the terminal shoots of regularly bearing trees had a smaller number of parenchymatous cells, and the fiber to parenchyma ratio was higher. The summer xylem, of which there was very little in the terminal shoots of shyly bearing trees and in the unfruitful shoots of biennially bearing trees, contained few parenchyma cells and relatively many fibers. The same relationship of cells in the spring wood did not appear to have this definite trend from the fruitful to the unfruitful shoots. Spring wood varied less throughout the different types

of shoots than did the summer wood. The quality of the early formed spring wood appeared to be related to the presence and utilization of food reserves. Summer wood seemed related to the current supply and accumulation of elaborated foods during the growth period.

That the character of the anatomical structure of the xylem may be markedly influenced by the chemical composition of the shoot was indicated by a study of the anatomy in Wealthy trees which represented wide extremes of growth character, fruitfulness, and composition (table II).

1. Shoots on which no blossom buds formed were obtained from an old, poorly vegetative, unfruitful, nitrogen starved tree. The xylem of such shoots was composed almost entirely of summer wood in which parenchyma was abundant and the ratio of fibers to parenchyma was the lowest of any samples examined. It has been reported (**5**) that samples similar to these have a high carbohydrate and low nitrogen composition.
2. Shoots on which many blossom buds formed, and which corresponded in external appearance to the fruitful terminal shoots of biennially bearing trees, were obtained from trees growing in pot culture. In these the xylem contained considerable summer wood in which the number of parenchyma cells was relatively high and the ratio of fibers to parenchyma was relatively low. It has been reported (**5, 6**) that such shoots have a relatively high carbohydrate and intermediate nitrogen content.
3. Shoots on which no blossom buds formed were taken from below a girdle on shaded trees growing in pots with a high nitrogen nutrient. The xylem of such shoots contained little summer wood and in it the number of parenchyma cells was small and the ratio of fibers to parenchyma was high. It has been reported (**6**) that samples similar to these have a low carbohydrate and high nitrogen content.

If the chemical composition of these types of shoots has been correctly reported, is it not further evidence that:

1. The fruitful terminal shoots of biennially bearing varieties (which have in the xylem a large amount of summer wood containing much parenchyma and few fibers) are of a relatively high carbohydrate and low nitrogen composition;
2. The terminal shoots of trees of regularly and shyly bearing varieties (which have in the xylem somewhat less summer wood and parenchyma and more fibers) are of somewhat lower carbohydrate and higher nitrogen composition;
3. The unfruitful terminal shoots of trees of biennially bearing varieties (which have in the xylem little summer wood, few parenchyma,

and many fibers) are of relatively low carbohydrate and high nitrogen composition?

A study of the anatomy of nursery whips also yielded further evidence. Some were defoliated and others girdled during midsummer. Sections of these and of untreated trees taken in late fall after growth had ceased showed that the relative amount of parenchyma in the last formed xylem was least in defoliated, next highest in untreated, and highest in girdled trees (table II). The relative carbohydrate content as observed in the pith of these trees is represented in the camera lucida diagrams of figure 6. The carbohydrate-nitrogen ratio has been found by HARVEY (**2**) to be least in defoliated, next highest in untreated, and highest in girdled shoots.

Nursery whips of different varieties were found to differ in number of parenchymatous cells and in the parenchyma to fiber ratio in the outer xylem. Of Spy, Fameuse, and Wealthy, Spy had relatively the lowest number of parenchyma cells, comparable to defoliated Northwestern. Fameuse was comparable to Northwestern untreated; Wealthy had relatively the greatest number of parenchymatous cells and was comparable to girdled Northwestern (table II). Of these same nursery trees the amount of starch and the secondary cell wall thickenings in the pith were greatest in Northwestern girdled and Wealthy untreated; were intermediate in Northwestern and Fameuse untreated; were least in Northwestern defoliated and Spy untreated (fig. 6).

If the relative number of parenchyma cells in the xylem and the relative amount of starch and secondary cell wall thickenings of the pith give an index of the amount of carbohydrates present in young trees, then the Wealthy, even in the nursery row, starts to accumulate carbohydrates fairly abundantly, Fameuse and Northwestern less so, and Spy least of all.

It may be significant that the age at which trees of these varieties ordinarily come into bearing is in the same order as the rate or degree to which these data indicate carbohydrates to be formed in the young nursery trees. The growth made by young trees of all varieties indicates that they tend to be of a higher nitrogen composition than older trees. As carbohydrates accumulate the tree makes less and less growth, a condition of fruitfulness finally resulting. Trees of those varieties which form carbohydrates most abundantly usually reach this condition first, depending upon cultural conditions. Similarly, when grown with a deficiency of nitrogen, they are often the first to reach, later in life, an unfruitful condition in which the character of growth produced is characteristic of an excessively high carbohydrate composition.

RESPONSE TO HIGH AND LOW NITROGEN NUTRIENT.—If trees of different varieties differ in formation and use of carbohydrate and nitrogen compounds, then the amount and quality of the growth produced by trees of

TABLE III

GROWTH OF ONE-YEAR OLD DWARF TREES IN POTS WITH HIGH AND LOW NITROGEN NUTRIENT. GROWN IN THE GREENHOUSE FROM JANUARY 30 TO MAY 14 AND THEN OUT-OF-DOORS UNTIL OCTOBER

	HIGH NITROGEN					LOW NITROGEN				
	WEALTHY	DUCHESS	MC-INTOSH	NORTH-WESTERN	SPY	WEALTHY	DUCHESS	MC-INTOSH	NORTH-WESTERN	SPY
Total growth (cm.)	75	48	137	175	83	29	24	58	55	77
Increase in weight (per cent.)	411	495	360	286	298	112	189	153	149	305
Fruitful	Yes	Yes	No	No	No	No	No	No	No	No

different varieties when grown under similar and controlled nitrogen nutrient conditions might be different.

It was found that potted trees of different varieties did not respond alike to similar nutrient conditions (table III). Under high nitrogen nutrient, Duchess and Wealthy trees were the only ones to form blossom buds the first season after potting. Trees of the other varieties were over-vegetative; that is, a relatively long, tapering, unfruitful growth was made. With low nitrogen nutrient conditions, Duchess and Wealthy trees were under-vegetative; that is, a short slender growth was made; the growth of McIntosh closely approached the fruitful character but no fruit buds were formed. Northwestern and Spy were still over-vegetative in growth type.

It is significant that under a high nitrogen nutrient Spy showed almost the least and Wealthy the greatest relative increase in weight; under low nitrogen nutrient Spy showed the greatest and Wealthy the least increase (table III). Spy increased in weight more in low nitrogen nutrient than in high; Wealthy and Duchess gained more weight in high nitrogen than in low.

In January of 1929 these trees were again potted, placed in the greenhouse, and allowed to grow without soil nutrient other than that contained in tap water. The amount of growth made under these conditions would seem fairly indicative of the quality or amount of previous seasons' reserves present in the trees. Of the trees previously grown in high nitrogen nutrient, Duchess and Wealthy made the most growth (total length), McIntosh and Northwestern less, and Spy least. This small amount of growth of Spy was apparently because of a deficiency of carbohydrate reserves formed in 1928. Of the trees which had been in low nitrogen nutrient in 1928, Spy made the most growth, Duchess and Wealthy least. This small amount of growth of Duchess and Wealthy was apparently because of a deficiency of nitrogen reserves formed in 1928.

Discussion

The recognition that the cause of the difference in fruiting performance between varieties may be related to differences in growth character as induced by differences in carbohydrate and nitrogen utilization by the trees provides a basis for planning cultural treatments. Growth type and carbohydrate and nitrogen composition can be altered in the orchard easily, practically, and in many instances economically, by such ordinary practices as pruning, the application of readily available nitrogenous fertilizers, and by other soil management programs. Since some varieties tend to be relatively higher in nitrogen and some relatively higher in carbohydrates, the same amount or kind of pruning, the same fertilizer applications, or the same soil management may have opposite effects when applied to different

varieties. The adaptation of cultural treatments to a variety must be based on the performance of that variety under the conditions and locality in which it is being grown. These can be only suggested here.

Trees of such varieties as Spy, Newell, and Golden Russett, upon reaching a bearing size, should be treated to increase the carbohydrate content in order to increase fruitfulness. Less of a nitrogenous fertilizer need be applied. Pruning should be of the thinning-out type to allow more sunlight to reach the leaves. Bending of branches to admit sunlight may be a practical means of inducing early bearing. This has been reported (**4**) to have increased the yields of 9-year-old Spy trees in New York.

By comparison, trees of such varieties as Duchess, Wealthy, or Transparent should be treated to avoid an excessively high carbohydrate content. This can be done by soil management practices which increase the available nitrogen of the soil; and by pruning, particularly of the heading-back type which gives the response of a lowered carbohydrate content or of an increased nitrogen content.

The present study suggests further evidence for the view that the fruiting performance of a tree is dependent upon the nutritional condition existing within it, rather than directly upon any specific environmental influence. It has emphasized the importance, as has been set forth by other workers, of a condition of balance between extremes in growth character and chemical composition of the trees as favoring fruitfulness. While genetic differences are perhaps the underlying causes for the differences in varietal behavior, they are expressed in orderly and more or less understandable physiological processes, a knowledge of which furnishes a basis for cultural treatment.

An interesting as well as an important fact that has come from this study is the relation between chemical composition, fruitfulness, growth character, growth rate and period, rate and period of xylem formation, presence or absence of summer wood, and cell distribution in the xylem. It may be that information can be obtained of one phase of plant performance by considering it in relation to others.

A study of the anatomical structure of the plant has been used as one means of measuring its physiological condition. This method of attack may prove of further value in the solution of some physiological problems, particularly in view of the present uncertainty of macrochemical methods to yield completely satisfactory results and also in view of the relative ease with which some seasonal nutritional changes can be detected anatomically.

The present study has emphasized the importance of the time and initial rate of growth in determining the fruitfulness of the resulting shoot. Further knowledge is needed of the chemistry of food reserves upon which growth in the spring is largely dependent.

Summary

1. The data presented give evidence that the relative position between the extremes of vegetativeness that trees of a variety normally occupy is correlated with the typical fruiting performance of trees of the variety. The degree of vegetativeness is closely related to or determined by the chemical composition of the tree, particularly as regards carbohydrate and nitrogen contents.

2. Of the samples analyzed, the starch and total carbohydrate contents and starch-nitrogen and total carbohydrate-nitrogen ratios were highest in fruitful terminal shoots of trees of biennially bearing varieties. They were next highest in terminal shoots of trees of regularly and shyly bearing varieties. They were lowest in the unfruitful terminal shoots of trees of biennially bearing varieties. (Nitrogen starved non-fruitful trees were not analyzed.)

3. Numerical expressions of chemical composition, particularly of carbohydrate-nitrogen ratios, are only relative because of limitations of present methods of macro-analyses.

4. Terminal growth was first initiated and proceeded more rapidly during the early part of the growing period in the fruitful than in the unfruitful terminal shoots of trees of biennially bearing varieties. Terminal growth of trees of regularly and shyly bearing varieties was intermediate in point of time of initiation and initial rate.

5. Accumulation of starch and formation of xylem were initiated earlier and proceeded more rapidly in the fruitful terminal shoots of trees of biennially bearing varieties than in the unfruitful terminal shoots. These were intermediate in the terminal shoots of trees of regularly and shyly bearing varieties.

6. The width of xylem in the terminal shoots of trees of regularly and shyly bearing varieties was intermediate between that of unfruitful and fruitful terminal shoots of trees of biennially bearing varieties.

7. Summer wood formation in the xylem was associated with carbohydrate accumulation. It was abundant in the xylem of fruitful terminal shoots of trees of biennially bearing varieties. It was less abundant in terminal shoots of trees of regularly and shyly bearing varieties and least abundant in unfruitful terminal shoots of trees of biennially bearing varieties.

8. A relatively large number of parenchymatous cells and a low fiber to parenchyma ratio in the xylem were associated with a high carbohydrate, low nitrogen composition. Conversely, a relatively small number of parenchymatous cells and a high fiber to parenchyma ratio in the xylem were associated with a low carbohydrate, high nitrogen composition.

9. Trees of different varieties propagated on dwarf stocks did not respond equally to similar nutrient conditions in amount and character of growth produced.

10. Fruitfulness was correlated with character of terminal growth, growth rate and period, rate and period of xylem formation, presence of summer wood, cell distribution in the xylem, and chemical composition.

11. The time and rate at which terminal growth, with its accompanying leaf formation, are initiated in the spring appeared to be highly correlated with the fruitfulness of the resulting growth.

12. The chemical composition of trees of a variety as indicated by the character of the terminal shoot growth may be used as an index to cultural needs.

13. The unfruitful growths of biennially bearing trees represent an extreme of composition. The fruitful growths represent an intermediate condition. A possible other extreme of samples from nitrogen-starved trees was not included.

Gratitude is extended to Dr. R. H. Roberts, who suggested this study and gave counsel throughout the course of the work, and to Dr. W. E. Tottingham and others of the plant chemistry department, University of Wisconsin, who gave helpful advice and kindly permitted the use of their laboratory for conducting the macrochemical analyses.

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DETERMINATION OF THE NITROGENOUS FRACTIONS IN VEGETATIVE TISSUE OF THE PEACH[1]

O. W. DAVIDSON AND J. W. SHIVE

Introduction

All vegetative parts of the peach contain a glucoside which may be hydrolyzed to form hydrocyanic acid, benzaldehyde, and glucose by the action of any enzyme, or enzymes, also present in the various tissues. The cyanogenetic glucoside or glucosides are not present in the same concentration in all parts of the plant. Thus in the fruit flesh it is found only in very minute amounts, although some investigators (**12**) have reported it to be absent from this part. Cyanogen compounds are known to occur in many plants, however, but they are supposed to be present only as glucosides. Nevertheless, WILLAMAN (**26**) and others have found in some plants what was regarded as either free hydrocyanic acid or hydrocyanic acid from a very unstable glucoside. Although no free hydrocyanic acid is known to exist in the peach, the cyanogenetic glucoside, or glucosides, are either more stable in dormant or slowly growing plants than in rapidly growing ones, or else their specific enzymes are relatively inactive in the slowly growing plants.

Because of the presence of a nitrogenous glucoside, or glucosides, peach tissue cannot be macerated in the preparation of an extract of soluble nitrogen without a consequent loss of nitrogen. Certain modifications in the procedure commonly employed for plant analyses are necessary, therefore, and the nitrogen contained in this glucoside must be determined and removed completely from an aqueous extract of peach tissues before the remaining nitrogenous fractions can be determined accurately.

In the present study an attempt has been made to modify the methods commonly employed for the determination of the nitrogenous fractions in plants in general, so that they may be used for investigations of these fractions in the tissues of the peach (*Prunus persica* Stokes).

The methods described are suitable for use with samples comprising 50 gm. or more of fresh plant material. Samples of this size have been found adequate to give representative results. Only aqueous extracts prepared from fresh plant material were regarded as suitable for use in this connection, since CHIBNALL (**4**) has shown that such extracts are more applicable than any others in a study of the nitrogen distribution in plants.

The investigation is admittedly incomplete in that it does not include a study of the composition of all parts of the peach plant sampled at various

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Physiology.

stages of growth. There may be parts of the plant that at times will require special handling previous to extraction, such as that indicated by ROBINSON (**14**).

Materials and methods

In these studies the stem material used consisted of twigs one year old or less taken from peach trees grown in an orchard or in sand cultures in a greenhouse. Only the non-woody portions of the root systems of peach trees grown in sand cultures were analyzed. The material consisted of the tender white ends of roots from which the cortex had not sloughed off, and 3–5 cm. of the older portions from which the cortex was missing.

PREPARATION OF TISSUE FOR EXTRACTION

In the preparation of an extract from peach tissue, it is necessary that the enzyme or enzymes which hydrolyze cyanogenetic glucosides be destroyed as early in the process as possible. Unless the material is handled quickly and precautions are taken to prevent or minimize the hydrolysis of the glucoside, some of the hydrocyanic acid liberated may be lost during the preparation of the extract. Before the enzyme is destroyed, however, the material must be prepared for aliquoting and extracting.

Accordingly the twigs were ground to shreds in a pencil sharpener (7). When entirely woody, the whole twig could be ground in this manner. In the case of actively growing shoots, however, the terminal 8–10 cm. of growth, and the short, slender side branches could not be ground satisfactorily in a pencil sharpener because of lack of rigidity in the tissue. Such material was therefore minced finely with pruning shears and then mixed thoroughly with the shredded material and aliquoted.

METHOD OF EXTRACTING

In studying the nitrogen distribution in the leaves of *Prunus laurocerasus*, ROBINSON (**14**) placed a small sample of minced leaves in a flask, added cold water, and then inserted a stopper fitted with tubes to permit a strong current of air to be passed through the contents. The aeration was started and the flask was heated until the contents reached boiling point, at which it was held for 20 minutes. Any hydrocyanic acid distilled over was collected in 5 per cent. sodium hydroxide contained in a flask immersed in an ice bath. By continuing the aeration for three hours after boiling, she presumably recovered any hydrocyanic acid liberated from the tissue before the emulsin was destroyed. Through this procedure she recovered half or more of the cyanide present in cherry laurel leaves. The remaining cyanogenetic nitrogen was recovered by the action of emulsin upon an aliquot of the aqueous extract.

Inasmuch as *P. laurocerasus* and *P. persica* are closely related species, it was expected that peach stem tissue subjected to this treatment would likewise yield a portion of the nitrogen contained in its cyanogenetic glucoside. The amounts of hydrocyanic acid recovered from samples of peach tissue boiled and aerated in this manner[2] varied widely. In the case of dormant and slowly growing peach stems no hydrocyanic acid was liberated. Samples comprised of the terminal 15–20 cm. of stems that were growing rather slowly in late August, however, liberated a very small amount of cyanogenetic nitrogen equivalent to only 0.0022 per cent. of their green weight. At the same time the stem tips of fast growing suckers taken from young trees yielded cyanogenetic nitrogen equivalent to 0.0145 per cent. of their green weights. During June, when the peach trees were growing most rapidly, a sample comprised of the terminal 15–20 cm. of rapidly growing stems liberated cyanogenetic nitrogen equivalent to as much as 0.0438 per cent. of the green weight. This sample contained a relatively large proportion of meristematic tissue rich in nitrogenous constituents. Nevertheless the unusually high proportion of hydrocyanic acid recovered in the distillate indicates that the cyanogenetic glucoside found in rapidly growing peach stems is less stable than that found in slowly growing stems, or else that the emulsin present in the former is more active than that in the latter.

It is interesting to note that no hydrocyanic acid was liberated when the leaves of rapidly growing peach stems were boiled and aspirated. The short period of time required for the preparation of a sample of the leaves before bringing them to a boil may have been too brief to allow an appreciable amount of hydrolysis of the cyanogenetic glucoside to take place.

VIEHOEVER, JOHNS, and ALSBERG (**22**) used various treatments for distilling the cyanogenetic nitrogen from *Tridens flavus*. Their results showed that no hydrocyanic acid, or at the most only a very small proportion, was liberated when the tissue was distilled without the addition of acid.

It is evident that a wide range of stability is exhibited by the various cyanogenetic glucosides found in plants. Indeed there is considerable indication of a variation in the stability of the cyanogenetic glucoside or glucosides found within a single plant (**26**).

The preliminary boiling under a reflux condenser recommended by ROBINSON was therefore omitted in the preparation of extracts from dormant and slowly growing peach stems, and from roots and leaves. In this case an aqueous extract was prepared by covering a 50-gm. aliquot of the finely minced plant material with boiling water in a large beaker, and

[2] In this work, hot instead of cold water was added to the samples in the distilling flasks in order to hasten the destruction of enzymes and to minimize the liberation of hydrocyanic acid from the tissue.

allowing the contents to boil for 20 minutes. Following this the tissue was transferred to "longcloth" and washed repeatedly, then placed in a mortar where the pieces minced with shears were pounded to a pulp. A further extraction was accomplished by boiling the tissue again for 10 minutes and then washing it several times, to make a volume of approximately 950 cc. This method is essentially the same as that adopted by VICKERY and PUCHER (**21**) for an investigation of the nitrogenous fractions in tobacco leaves. It has been studied recently by DAVIDSON, CLARK, and SHIVE (**6**) for use with a number of plants, including the peach.

When actively growing peach stems were extracted, it was necessary to use the preliminary distillation and aspiration recommended by ROBINSON. For this purpose the sample was placed in a 2-liter Florence flask and covered with 400 to 500 cc. of boiling water. After the aspiration had been continued for three hours, the sample was extracted as described in the preceding paragraph.

Owing to the bulkiness of the shredded peach stem tissue, samples heavier than 50 gm. were seldom used, and samples weighing from 80 to 100 gm. were always extracted with about 1900 cc. of water in order to insure a thorough removal of the soluble nitrogen present. Since minced peach roots are much less bulky than minced or shredded peach stems, root samples heavier than 50 gm. could be extracted conveniently with 1 liter of water.

A peach extract obtained in this manner contains very little nitrogen that can be coagulated by heating with very dilute acetic acid. Nevertheless the extract, having a volume of approximately 900 to 950 cc., was heated to boiling in a large beaker, 1.5 cc. of 10 per cent. acetic acid was added, and the solution was allowed to boil for 1 minute. The hot extract then was filtered through paper pulp in a Buchner funnel with the aid of suction.

In a previous investigation (**6**), the amounts and distribution of soluble nitrogen in representative aliquots of peach stems were compared after extraction with boiling water as just described, and after grinding in a mortar with the aid of sand, followed by extraction with cold water. By the latter method the glucoside probably was exposed to the active enzyme for more than an hour longer than it was by the boiling method. During this period of maceration in the mortar, 11.66 per cent. of the cyanogenetic nitrogen present either was lost or was combined in some stable compound that did not permit its release in the subsequent treatments. ALSBERG and BLACK (**1**), working with *Prunus virginiana* and *Andropogon sorghum,* recovered less hydrocyanic acid after a period of maceration than by the distillation of fresh comminuted leaves.

CYANOGENETIC NITROGEN

An analysis of the various nitrogenous fractions in an extract of peach tissue presents little difficulty after the complete removal of cyanogenetic

nitrogen has been effected. Indeed for the analysis of an extract free of this form of nitrogen, the methods employed by NIGHTINGALE *et al.* (**9, 10**), TIEDJENS and ROBBINS (**19**), TIEDJENS and BLAKE (**20**), and others (**5**) may be used with only slight modifications.

The quantitative recovery of cyanogenetic nitrogen in peach tissue extracts, however, has presented considerable difficulty. A review of the literature dealing with methods for determining this form of nitrogen in plants has revealed a variety of procedures, none of which is entirely satisfactory.

DETERMINATION BY ACID HYDROLYSIS.—The hydrolysis of amygdalin by acids and by emulsin has attracted the attention of chemists for many years. AULD (**2**), WALKER and KRIEBLE (**25**), and later KRIEBLE (**8**), have made special studies of the methods and products of the hydrolysis of amygdalin. These investigations have shown that the nitrogen in amygdalin is converted readily to ammonium sulphate by the action of concentrated sulphuric acid. When treated with dilute sulphuric acid, however, the hydrolysis takes a different course, forming l-mandelonitrile, from which hydrocyanic acid is liberated slowly and incompletely. These studies have shown clearly that the hydrolysis of amygdalin, with the subsequent liberation of hydrocyanic acid, is accomplished more rapidly and more completely by the action of emulsin than by that of acids.

ALSBERG and BLACK (**1**) concluded that all of the hydrocyanic acid that could be obtained from the leaves of *Prunus virginiana* was liberated during hydrolysis by boiling with 5 per cent. sulphuric acid for 4 hours, whereas that recovered from *Andropogon sorghum* and *Panicularia nervata* was liberated during hydrolysis for 1 hour with the same concentration of acid. This conclusion, however, was based upon the assumption that all of the cyanogenetic nitrogen present in the glucosides was liberated when further acid hydrolysis failed to release any more hydrocyanic acid, an assumption that has been shown (**26**) to be unjustified.

It was considered here that information concerning the extent to which hydrocyanic acid is liberated from extracts of peach tissue during hydrolysis with sulphuric acid might be of considerable importance. Thus if it were found that this form of nitrogen could be removed quantitatively by such treatment, then, by subtraction, Kjeldahl determinations before and after hydrolysis would reveal the amount of cyanogenetic nitrogen present. Furthermore, if this fraction could be removed during the 2.5-hour hydrolysis with 5 per cent. sulphuric acid used in the determination of amide nitrogen, the analysis would be simplified by the elimination of one procedure.

Accordingly aliquots of an extract prepared from peach stems were hydrolyzed by boiling for different lengths of time under condensers of

the Hopkins type with two concentrations of sulphuric acid. The amounts of hydrocyanic acid liberated were determined by a comparison of the amounts of total nitrogen in the aliquots before and after hydrolysis. Similar aliquots of the same extract also were hydrolyzed with emulsin, following a method that will be described later. The results obtained, together with data showing the behavior of c.p. amygdalin when hydrolyzed with dilute sulphuric acid and with emulsin, are presented in table I. It is apparent that, during acid hydrolysis, the behavior of the cyanogenetic glucoside in a peach stem extract is very similar to that of amygdalin. It is also apparent that the decomposition of the glucoside by hydrolysis with dilute sulphuric acid is far from complete. Nevertheless duplicate aliquots, after hydrolysis for the same time and with the same concentration of acid, always yielded similar amounts of nitrogen. This fact would seem to indicate that, under similar conditions of hydrolysis, the decomposition of the glucoside stops at a fairly definite stage.

TABLE I

Comparison of amounts of hydrocyanic acid nitrogen liberated from aliquots of a peach stem extract and from a 1% solution of amygdalin by hydrolysis with dilute sulphuric acid and with emulsin

Treatment	Total nitrogen	Yield of HCN–N	Recovery or loss of HCN–N
	gm.	*gm.*	%
100 cc. of stem extract, Kjeldahlized	0.00860		
100 cc. of stem extract, hydrolyzed with emulsin	0.00555*	0.00309	100†
100 cc. of stem extract, hydrolyzed 2.5 hours with 5% H_2SO_4	0.00649	0.00211	69
100 cc. of stem extract, hydrolyzed 5 hours with 5% H_2SO_4	0.00607	0.00253	82
100 cc. of stem extract, hydrolyzed 5 hours with 10% H_2SO_4	0.00656	0.00203	66
100 cc. of 1% amygdalin, Kjeldahlized	0.02813		
100 cc. of 1% amygdalin, hydrolyzed with emulsin		0.02810	100
100 cc. of 1% amygdalin, hydrolyzed 2.5 hours with 5% H_2SO_4	0.00778	0.02055	73

* The amount HCN–N recovered by hydrolysis with emulsin is taken as 100% of that present in the aliquot, although the actual recovery from c.p. amygdalin was 99.89%.

† This figure does not include any of the nitrogen added by emulsin.

Determination by enzymatic hydrolysis.—Willaman (**26**), after obtaining unusually low yields of hydrocyanic acid from amygdalin by

means of hydrolysis with 5 per cent. sulphuric acid, abandoned this procedure for the analysis of plant tissues. He therefore chose emulsin as an hydrolytic agent and recovered as much as 98.5 per cent. of the theoretical amount of cyanide nitrogen present in samples of amygdalin when the enzyme was allowed to operate over a period of 24 hours.

In the more recent work of ROBINSON (**14**), the cyanogenetic glucosides in *Prunus laurocerasus,* as well as in *Andropogon sorghum,* were hydrolyzed by emulsin and the hydrocyanic acid formed was removed by aspiration for 3 hours and collected in 5 per cent. sodium hydroxide. This method has the advantage of being much more rapid than that used by WILLAMAN, since the removal of hydrocyanic acid favors a more complete hydrolysis of the glucoside. This may be anticipated from the work of AULD (**2**), who has shown that the hydrolysis of amygdalin by emulsin may not go to completion in the presence of the hydrolytic products. KRIEBLE (**8**) has shown also that emulsin has synthetic properties in the presence of hydrocyanic acid and benzaldehyde.

It is of interest to note that none of the investigators studying cyanogenesis in plants recognized any need for adjusting the solution containing the glucoside to a reaction favorable to the hydrolytic activity of emulsin. Although this enzyme is active over a fairly wide range of pH values, its optimum range is comparatively narrow. VULQUIN (**23**) found that regardless of the agents used to adjust the reaction of the solution, and regardless of the quality of the emulsin used, the maximum cleavage of amygdalin occurs when the hydrogen ion concentration is between pH 5.2 and 5.7. WILLSTÄTTER and CSANYI (**27**) concluded that a reaction between pH 5.0 and 6.5 is favorable to the hydrolysis of amygdalin by emulsin, and that the optimum reaction is approximately pH 6.

Inasmuch as the reaction of extracts of peach tissue prepared as described previously was usually about pH 4.6 to 4.7, it was considered advisable to determine the reaction at which such extracts yield the maximum amounts of hydrocyanic acid when hydrolyzed with emulsin. For this purpose a 1-liter extract was prepared from 50 gm. of peach stems (var. Cumberland), and duplicate 100-cc. aliquots of this were adjusted to the reactions shown in table II. The amounts of cyanogenetic nitrogen present were then determined by the method recommended by ROE (**15**). Thus 0.05[3] gm. of emulsin was added to each aliquot contained in a 300-cc. Florence flask, which was then closed by inserting a rubber stopper containing aspiration tubes sealed by clamps placed on the rubber connections.

[3] In later analyses, 0.02 gm. of emulsin was found adequate for such extracts. Since some preparations of emulsin have a high content of nitrogen, a known amount of the enzyme should always be added and an excess avoided when the extract is to be partitioned for the determination of other nitrogen fractions.

The aliquots were warmed to 40°–50° C. for 15 to 30 minutes, after which each flask was connected to a correspondingly numbered flask containing 100 cc. of 5 per cent. sodium hydroxide. The flasks were arranged in a series and a strong current of air was drawn through them for 4 hours. The cyanide collected in the sodium hydroxide flasks was titrated with N/100 silver nitrate, using 10 drops of 10 per cent. potassium iodide as an indicator. At the end of the period of aspiration the reactions of the various aliquots were determined again and were found to have changed in all but one instance. This change was due probably to the fact that the initial pH readings were taken before equilibrium in the extract had been established.

The amounts of cyanogenetic nitrogen obtained from the various aliquots during this experiment are shown in table II. The maximum recovery, that at pH 5.7, is expressed as 100 per cent. for comparison with the recovery at other reactions. That the amount recovered at pH 5.7 may be very close to 100 per cent. of the cyanogenetic nitrogen present in the extract is indicated by the fact, as shown in table I, that 99.89 per cent. of the nitrogen present in aliquots of a 1 per cent. solution of amygdalin adjusted to this reaction was recovered under similar conditions.

TABLE II

COMPARISON OF EFFECTS OF HYDROGEN ION CONCENTRATION OF EXTRACT UPON AMOUNTS OF CYANOGENETIC NITROGEN RECOVERED FROM PEACH STEMS

INITIAL PH	FINAL PH	CYANOGENETIC NITROGEN RECOVERED	
		GRAMS PER 50 GM. OF TISSUE	PERCENTAGE RECOVERY
		gm.	%
5.0	5.1	0.02381	95.9
5.0	5.0	0.02381	95.9
5.5	5.3	0.02410	97.1
5.5	5.3	0.02410	97.1
6.0	5.7	0.02497	100.0
6.0	5.7	0.02468	
6.5	6.0	0.02410	97.1
6.5	6.1	0.02381	95.9
7.0	6.6	0.02265	87.2
7.0	6.5	0.02322	93.5

Another experiment similar to the one just described was carried out using a 1-liter extract prepared from 50 gm. of stem tissue taken from a different tree (*P. kansuensis* × *P. persica* hybrid). One hundred cc. aliquots of this extract were adjusted to reactions ranging from pH 4.0 to 7.0, as shown in table III. After standing for 4 hours the reactions showed a change of from 0.1 to 0.5 of a pH unit. The solutions were readjusted to the desired reactions, therefore, 0.05 gm. of emulsin added to each, and the

cyanogenetic nitrogen determined as in the preceding experiment. Subsequent changes in the reactions of the solutions were very slight. The amounts of cyanogenetic nitrogen found are presented in table III, where the maximum recovery is expressed as 100 per cent. for comparative purposes.

TABLE III

COMPARISON OF EFFECTS OF HYDROGEN ION CONCENTRATION OF EXTRACT UPON AMOUNTS OF CYANOGENETIC NITROGEN RECOVERED FROM PEACH STEMS

pH	Cyanogenetic nitrogen recovered	
	Grams per 50 gm. of tissue	Percentage recovery
	gm.	%
4.0	0.02621	63.8
4.5	0.03717	90.5
5.0	0.03891	94.7
5.5	0.04109	100.0
6.0	0.04029	98.1
6.5	0.03731	90.8
7.0	0.01713	41.7

From these two experiments it is apparent that the optimum reaction for the enzymatic hydrolysis of the cyanogenetic glucosides in an extract of peach tissue is approximately pH 5.5 to 5.7. It is apparent also that a reaction between pH 5.5 and 6.0 is satisfactory for most analytical purposes. This range of hydrogen ion concentrations is in good agreement with those found by VULQUIN (**23**) and by WILLSTÄTTER and CSANYI (**27**) for enzymatic hydrolysis of amygdalin.

In this connection it is of interest to note that the reaction of an extract of peach stems prepared without the addition of any acid was found to be approximately pH 5.3. Such a reaction, of course, is too low for the maximum recovery of the cyanogenetic nitrogen present. This does not mean, however, that the emulsin naturally present in peach tissues functions at pH 5.3. Cells in the phloem of the peach range in reaction from pH 5.4 to approximately pH 6.5, whereas most of the cells in the xylem have reactions ranging from pH 4.2 to 4.4. The extract of peach stems just described represents a composite sample of the soluble constituents of all tissues present, and its reaction should therefore be intermediate between the highest and the lowest reactions found in the cells.

It is apparent, therefore, that for the complete recovery of the cyanogenetic nitrogen from extracts of peach tissue, as well as from solutions of amygdalin, an enzymatic hydrolysis should be employed. It is evident also that this hydrolysis should be conducted at a reaction between pH 5.5 and 6.0, and that the hydrocyanic acid formed should be removed rapidly.

Ammonium nitrogen

Robinson (14) displaced ammonia from extracts of *P. laurocerasus* with a saturated solution of borax during distillation *in vacuo* at 60° C., after the method of Watchorn and Holmes (24). Since it has been shown (16, 19) that when 0.5 per cent. sodium hydroxide was substituted for a saturated solution of sodium carbonate in the method of Sessions and Shive (16), slightly but consistently larger amounts of ammonia were recovered from plant extracts, it was anticipated that the use of borax would not displace all of the ammonium nitrogen present in peach extracts. Tiedjens (18) found that the amount of ammonium nitrogen recovered by aspiration with 0.5 per cent. sodium hydroxide corresponded to the amount recovered by electrodialysis.

Aliquots of a peach stem extract were analyzed for ammonium nitrogen, both by the method of Watchorn and Holmes and by the method of Sessions and Shive, using 0.65 per cent. sodium hydroxide.[4]

When the results of the two methods were compared (table IV), the difference in the amounts of ammonium nitrogen recovered was far greater than was expected. It was obvious that some form of nitrogen, not normally present as ammonium, was being liberated by the treatment with dilute sodium hydroxide but not by the treatment with borax.

TABLE IV

Comparison of amounts of ammonium nitrogen recovered from peach stem extracts in the presence of cyanogenetic nitrogen and ferrous sulphate

Treatment	Nitrogen recovered from 50 gm. of tissue
	gm.
1. Aspirated with 0.65% NaOH in presence of cyanogenetic nitrogen	0.0363
2. Aspirated with 0.65% NaOH after removal of cyanogenetic nitrogen	0.0064
3. Distilled *in vacuo* with a saturated solution of borax in presence of cyanogenetic nitrogen	0.0024
4. Distilled *in vacuo* with a saturated solution of borax after removal of cyanogenetic nitrogen	0.0023
5. Aspirated with 0.65% NaOH in presence of cyanogenetic nitrogen plus 5 mg. of $FeSO_4 \cdot 7H_2O$	0.0200
6. Aspirated with 0.65% NaOH in presence of cyanogenetic nitrogen plus 500 mg. of $FeSO_4 \cdot 7H_2O$	0.0076

[4] In preliminary work it was found that 0.65 per cent. sodium hydroxide displaced very consistent amounts of ammonia from peach extracts, whereas the use of 0.5 per cent. sodium hydroxide did not always give good agreement among duplicates.

In order to obtain further information regarding this point, the aliquots used were made neutral to litmus, adjusted to a reaction between pH 5.5 and 6.0, and the amount of cyanogenetic nitrogen present determined. No cyanogenetic nitrogen was liberated after the treatment with dilute sodium hydroxide, and only 68 per cent. of the amount previously found to be present was recovered from aliquots treated with borax. Moreover, the data shown in table V indicate that the increase in the amount of nitrogen recovered during aspiration with 0.65 per cent. sodium hydroxide does not account for all of the cyanogenetic nitrogen present in the extract. The results show also that both of the methods used for the determination of ammonium nitrogen in the presence of cyanogenetic nitrogen either caused a loss of the same amount of the latter, or caused the same amount of the glucoside to be affected in such a manner that it was stable toward emulsin.

As a means of studying the behavior of the cyanogenetic glucoside at room temperatures, solutions of amygdalin were aspirated with different concentrations of alkali and were found to liberate varying amounts of the nitrogen present. The results showed that the nitrogen in amygdalin may be wholly or partially converted to ammonia during hydrolysis with 0.65 per cent. sodium hydroxide at room temperatures. It was noticed, however, that the first one or two flasks in a series (those nearest to the suction pump) always liberated more ammonia than did any of the others. The

TABLE V

SUMMARY OF FACTORS AFFECTING RECOVERY OF CYANOGENETIC NITROGEN FROM PEACH STEM EXTRACTS

TREATMENT	GRAMS IN 50 GM. OF STEMS
	gm.
1. Cyanogenetic nitrogen recovered from initial aliquots by hydrolysis with emulsin	0.0482
2. Cyanogenetic nitrogen recovered by aspiration with 0.65% NaOH	0.0299
3. Cyanogenetic nitrogen recovered after hydrolysis of (2) by emulsin	0.0000
4. Cyanogenetic nitrogen tied up during aspiration with 0.65% NaOH	0.0153
5. Cyanogenetic nitrogen recovered by hydrolysis with emulsin following displacement of ammonia by borax	0.0330
6. Cyanogenetic nitrogen tied up during displacement of ammonia by borax	0.0152
7. Nitrogen in addition to that from amides recovered after hydrolysis of (3) with 5% H_2SO_4 for 2.5 hours (emulsin blank deducted)	0.0159

erratic results thus obtained with amygdalin were not typical of the behavior of peach extracts during similar treatment.

The failure to obtain complete recovery of cyanogenetic nitrogen following ammonia determinations suggested that ferro- or ferricyanides might be formed by a reaction between the soluble iron compounds present and any cyanide liberated from the glucoside in an alkaline solution. The nitrogen involved in such a reaction would of course be combined in an undissociated complex, from which it could be recovered neither by alkaline nor by enzymatic hydrolysis. The presence of such compounds in peach extracts, however, could not be established definitely. Nevertheless, the addition of ferrous sulphate to an extract before aspiration with 0.65 per cent. sodium hydroxide always resulted in the recovery of less nitrogen than was obtained by similar treatment without the addition of the iron salt. In table IV it will be observed that, when 500 mg. of ferrous sulphate were added to an extract, the amount of nitrogen recovered corresponded closely with, but was somewhat higher than, the amount of ammonium nitrogen found in the extract, indicating that practically all of the cyanogenetic nitrogen was combined in some way with the iron salt.

Referring to table V again, it is observed that the cyanogenetic nitrogen not recovered from a peach extract during aspiration with 0.65 per cent. sodium hydroxide was recovered quantitatively after hydrolysis by boiling for 2.5 hours with 5 per cent. sulphuric acid. This demonstrated that no cyanogenetic nitrogen was lost during ammonia determinations, and showed that it was not combined as an iron cyanide complex, since the latter during hydrolysis with dilute sulphuric acid should form hydrocyanic acid instead of an ammonium salt.

It is apparent, therefore, that ammonium nitrogen in peach extracts should be determined by aspiration with sodium hydroxide only after the removal of cyanogenetic nitrogen. Also, since the use of 0.65 per cent. sodium hydroxide permits a more nearly complete displacement of ammonium nitrogen from the extracts, it is preferable to either borax or sodium carbonate for this purpose, especially since the addition of these substances produces a high salt concentration which often interferes with aliquoting for amino nitrogen.

Total soluble nitrogen

Following the removal of cyanogenetic and ammonium nitrogen, the aliquots in which these fractions were determined were neutralized, made faintly acid by the addition of a drop of acetic acid, and then evaporated to dryness. The amount of nitrogen remaining in the aliquots was estimated by Kjeldahlization as modified to include nitrates. The sum of cyanogenetic, ammonium, and Kjeldahl values gave the amount of total soluble nitrogen present.

Amide nitrogen

It was shown in table I that, when a peach extract was hydrolyzed with 5 per cent. sulphuric acid for 2.5 hours, the procedure usually employed in the determination of amides, only about 75 per cent. of the cyanogenetic nitrogen present was liberated as hydrocyanic acid. In table V it was shown that, when the cyanogenetic nitrogen was not removed completely before the amide hydrolysis, the values obtained for the latter fraction were high. It is apparent, therefore, that the cyanogenetic nitrogen should be removed before amide nitrogen is determined.

Since emulsin contains a high percentage of nitrogen, it was realized that either this substance must be removed after the hydrolysis of the glucoside, or the amounts of nitrogen added by it to the amide, basic, amino, total soluble, etc., fractions must be determined. When, however, attempts were made to precipitate emulsin and to filter it from extracts, its complete removal was never satisfactorily accomplished. Robinson (**14**) likewise reported inability to remove by precipitation the nitrogen added by emulsin.

In order to determine how the addition of emulsin to a peach extract would affect the various nitrogenous fractions studied, duplicate 0.0500-gm. samples of the enzyme were subjected to precisely the same procedure as were those used in the analysis of the extracts. The results presented in table VI show that the amounts of emulsin ordinarily added to an extract increase appreciably only the total soluble, amide, total amino, and basic nitrogen fractions. Hence it is obvious that, when the nitrogenous composition of the emulsin preparation is known and when carefully weighed amounts of the latter are used, the nitrogen thus added to the above fractions of the extract may be deducted.

During the present study the extent to which the nitrogenous composition of different preparations of emulsin may vary was not investigated,

TABLE VI

Distribution of nitrogen in 0.0500 gm. samples of emulsin used in hydrolysis

Fraction	Amount of nitrogen found	
	gm.	%
Total N	0.00559	11.180
Cyanogenetic N	None	None
Ammonium N	0.00006	0.012
Amide N	0.00099	0.980
Humin N	None	None
Total amino N	0.00112	2.232
Mono amino N	0.00008	0.016
Basic N	0.00168	3.360

since material from only a single preparation was used. Nevertheless it may be anticipated from the studies of WILLSTÄTTER and OPPENHEIMER (**28**) that different preparations of emulsin might vary in their composition.

The following method was therefore adopted for the estimation of amide nitrogen in peach extracts: After the removal of two 100-cc. aliquots used for cyanogenetic, ammonium, and total soluble nitrogen determinations, the remaining 800 cc. of a 1-liter extract were evaporated to 200 cc. and divided into two equal portions. Since the remaining soluble nitrogen fractions in the extract were present only in very small amounts, their estimation in concentrated aliquots avoided the use of an unnecessarily large aliquot factor. Cyanogenetic nitrogen was then removed from each of these aliquots as described previously. Sulphuric acid sufficient to give a concentration of 5 per cent. was added and the amide nitrogen present was hydrolyzed to ammonium nitrogen by boiling under condensers for 2.5 hours. The aliquots were then neutralized and the total ammonium nitrogen present determined by aspiration with 0.65 per cent. sodium hydroxide. The increase in nitrogen recovered over that found previously in the ammonia was amide nitrogen.

HUMIN NITROGEN

Following the removal of amide nitrogen, the aliquots were neutralized and the acid insoluble humin precipitate formed during the amide hydrolysis was filtered off. The precipitate was washed thoroughly with hot distilled water, dried, and Kjeldahlized. The nitrogen found in this precipitate is referred to as humin nitrogen.

NITRATE NITROGEN

Nitrate nitrogen was determined by the method of SESSIONS and SHIVE (**16**) on aliquots free of cyanogenetic nitrogen. Since 0.65 per cent. sodium hydroxide was used for the reduction with Devarda's alloy and for the subsequent displacement of ammonia, cyanogenetic nitrogen, if present, would be converted partially to ammonia and would therefore give high values for the nitrate fraction.

Accordingly this fraction was sometimes estimated in the same aliquots in which the ammonium nitrogen had been determined, but the presence of Devarda's alloy often made it inconvenient to use these samples for the Kjeldahlization of the remaining soluble nitrogen. It was found very convenient, however, to estimate nitrates in 25 cc. of the filtrate from the humin precipitate after it had been made to a volume of 100 cc.

BASIC NITROGEN

The phosphotungstic acid method of OSBORNE and HARRIS (**11**) was employed to precipitate the basic nitrogen present in 50-cc. aliquots of the

filtrate from the humin precipitate. The basic precipitate was not washed with a dilute solution of the precipitating reagent, since it was found that such treatment often dissolved some of the precipitate and caused poor agreement between duplicates. The aliquots were filtered through small sized filter paper and the precipitates allowed to drain overnight. Inasmuch as the filtrate contained only a very low percentage of nitrogen, the amount of non-basic nitrogen retained in the precipitate by this method of handling was slight.

Amino nitrogen

Amino nitrogen was always estimated by the Van Slyke method on 10-cc. portions of the filtrate from the humin precipitate, and on similar portions of the filtrate from the basic precipitate. With the exception of extracts prepared from young, actively growing trees, the amounts of amino nitrogen found in peach extracts of the concentrations used in this study were very small. Because of this, a gas burette from a micro-Van Slyke apparatus was fitted on a macro-apparatus in order to permit the more accurate reading of the gas evolved.

Total nitrogen

It was anticipated from the work of Ranker (13) that the Kjeldahl method, modified to include nitrates, would not recover all of the nitrogen present when determinations were made on green tissue containing nitrates. Strowd (17) has shown also that appreciable amounts of nitrates are recovered by Kjeldahl determinations even without the addition of reducing agents. Hence it seemed inadvisable to use the ordinary Kjeldahl method without the modification to include nitrates, and then to add to the amount of nitrogen recovered any nitrate nitrogen found by separate determinations to be present. It is evident that, for the accurate recovery of total nitrogen in the presence of nitrates, only dried plant material should be used; nevertheless the work of Robinson (14) indicates that a loss of nitrogen as hydrocyanic acid may take place during the drying of peach tissue.

In accordance with these considerations, different lots of peach tissue, some containing nitrates and some free of nitrates, were analyzed for total nitrogen by the use of a sulphuric-salicylic acid mixture and zinc to fix and reduce nitrates. Samples consisting of 8 gm. of green tissue were weighed rapidly but carefully in triplicate, and covered immediately with the sulphuric-salicylic acid mixture. Other aliquots of the same tissues were placed in an oven operated at 103° to 105° C. for one-half hour, after which the temperature was lowered to 65°–70° C. until the material was dry. These dried samples were ground in a drug mill, heated to 70° C. for one hour, and then cooled in a desiccator. Total nitrogen determinations were run on 1-gm. portions of the dried material, using the same procedure as

that used on the green tissue. A comparison of the results is shown in table VII.

TABLE VII

COMPARISON OF QUANTITIES OF TOTAL NITROGEN, EXPRESSED AS PERCENTAGE OF DRY WEIGHT, RECOVERED BY KJELDAHL DETERMINATIONS ON GREEN AND DRY TISSUE, WITH AND WITHOUT THE PRESENCE OF NITRATES

TISSUES USED	PECENTAGE OF NITRATE-NITROGEN PRESENT IN DRY TISSUE	NITROGEN RECOVERED FROM GREEN TISSUE	NITROGEN RECOVERED FROM DRY TISSUE
	%	%	%
Stems	0.000	1.695	1.718
Stems	0.000	1.498	1.524
Stems	0.000	1.260	1.267
Stems	0.041	1.182	1.281
Roots	0.000	4.026	3.931
Roots	0.023	3.750	3.694
Roots	0.944	3.311	3.675
Roots	2.518	3.439	3.974

It is obvious that peach tissue loses little if any of its nitrogen during the drying process. There is some indication of a slight loss of nitrogen from root tissue while drying, but there is no evidence of any loss occurring from stem tissue during similar treatment. The tender, white, non-woody portions of the peach roots used in these analyses contained considerably more of the cyanogenetic glucoside on a dry weight basis than did the stems. At the same time it is possible that the glucoside contained in peach roots is less stable than that found in the stems. WILLAMAN (**26**) believes that sorghum does not contain all of its cyanogenetic nitrogen in the form of dhurrin, and that at times a part, or all, of this form of nitrogen may exist in some less stable compound.

Peach roots may also contain a high percentage of nitrates, all of which may not be recovered by the modified Kjeldahl determination, unless the tissue is thoroughly dry before the sulphuric-salicylic acid mixture is added, and unless the digestion is conducted very slowly.

DISTRIBUTION OF NITROGEN

The amounts and distribution of nitrogen in peach trees vary tremendously with the conditions under which they are grown and with the activity of the plants at the time of sampling. In order to illustrate these variations, the percentages of the different nitrogenous fractions found in seedlings of Early Crawford, sampled while still growing in a greenhouse on November 1, are compared in table VIII with similar fractions found in the variety Cumberland sampled in the orchard on March 16. Although

TABLE VIII

NITROGENOUS FRACTIONS IN PEACH STEMS AND ROOTS EXPRESSED AS PERCENTAGE OF GREEN AND DRY MATTER, AND AS PERCENTAGE OF TOTAL NITROGEN

	STEMS FROM DORMANT TREES IN ORCHARD 3/16/33			ROOTS* FROM GROWING TREES IN GREENHOUSE 11/1/32			STEMS* FROM GROWING TREES IN GREENHOUSE 11/1/32		
	GREEN	DRY	TOTAL N AS 100%	GREEN	DRY	TOTAL N AS 100%	GREEN	DRY	TOTAL N AS 100%
	%	%	%	%	%	%	%	%	%
Total N	0.4182	1.2810	100.00	0.2839	3.6750	100.00	0.5042	0.9761	100.00
Total organic N	0.3927	1.2029	93.90	0.2080	2.6922	73.27	0.4910	0.9506	97.38
Protein N	0.2173	0.6657	51.96	0.1539	1.9920	54.22	0.3768	0.7295	74.73
Soluble organic N	0.1754	0.5372	41.94	0.0541	0.7002	19.05	0.1142	0.2211	22.65
Cyanogenetic N	0.0136	0.0417	3.25	0.0048	0.0621	1.69	0.0446	0.0863	8.85
Amide N	0.0450	0.1378	10.76	0.0144	0.1864	5.07	0.0126	0.0244	2.50
Humin N	0.0133	0.0407	3.18	0.0012	0.0155	0.42	0.0070	0.0136	1.39
Total amino N	0.0773	0.2368	18.48	0.0276	0.3572	9.72	0.0326	0.0631	6.47
Basic-free amino N	0.0566	0.1734	13.53	0.0152	0.1967	5.35	0.0154	0.0298	3.05
Basic N	0.0468	0.1433	11.19	0.0168	0.2174	5.92	0.0320	0.0620	6.35
Ammonium N	0.0121	0.0371	2.89	0.0030	0.0388	1.06	0.0132	0.0256	2.62
Nitrate N	0.0134	0.0410	3.20	0.0729	0.9436	25.67	none	none	none
Other N	0.0001	0.0003	0.04	0.0017	0.0220	0.60	0.0026	0.0049	0.51

* Owing to the methods of analysis used with these two samples, the values presented for cyanogenetic, ammonium, and amide nitrogen may not be absolutely correct. Nevertheless any inaccuracies present are believed to be of minor importance.

no attempt has been made to ascertain the extent of varietal differences, the data show a wide range in the proportions of nitrogen found in the cyanogenetic, amide, amino, basic, and nitrate fractions.

Nitrates, for example, are not usually found in stems and leaves of peach trees grown in orchards in New Jersey, but they are sometimes present in small amounts in the tops of trees grown in sand cultures and supplied with nutrient solutions having high reactions and containing an abundance of this form of nitrogen. As shown in table VIII, the small non-woody roots of peach trees grown in sand cultures may contain considerably more nitrate than soluble organic nitrogen. When grown under orchard conditions, however, such roots seldom contain much nitrate nitrogen.

It is apparent that peach trees may exhibit wide differences in the distribution of their nitrogen. This is probably true for all plants. The significance of such differences in the peach cannot be considered here but will be dealt with in another publication.

Summary

1. No hydrocyanic acid was liberated when dormant or slowly growing peach stems were minced rapidly and boiled according to the method recommended by ROBINSON (**14**). With the same method of treatment the terminal portions of moderately or rapidly growing peach stems liberated very small to very appreciable quantities of hydrocyanic acid.

2. The optimum reaction for the enzymatic hydrolysis of the cyanogenetic glucosides in extracts of peach tissues is approximately pH 5.5 to 5.7. A reaction between pH 5.5 and 6.0 is satisfactory for most analytical purposes.

3. The cyanogenetic nitrogen present in peach extracts never was completely liberated during hydrolysis by boiling with various concentrations of sulphuric acid for different lengths of time.

4. During aspiration with 0.65 per cent. sodium hydroxide in the determination of ammonium nitrogen by the method of SESSIONS and SHIVE (**16**), cyanogenetic nitrogen may be more or less completely hydrolyzed to ammonium nitrogen. Because of this it was found necessary to remove cyanogenetic nitrogen as a first step in the analytical procedure in order to avoid inaccuracies in the determination of ammonium and amide nitrogen.

5. The addition of a soluble iron salt to a peach extract was found to prevent almost completely the hydrolysis of cyanogenetic nitrogen to ammonium nitrogen in an alkaline solution.

6. Considerably more ammonium nitrogen may be recovered from extracts of peach tissues by aspiration with 0.65 per cent. sodium hydroxide at room temperatures by the method of SESSIONS and SHIVE than by distillation *in vacuo* at 60° C. with a saturated solution of borax according to the method of WATCHORN and HOLMES.

7. When precautions were taken to destroy quickly the emulsin present in green peach tissues, no loss of nitrogen occurred during the drying of stem material. Under similar conditions of drying, root material lost a slight but not appreciable amount of nitrogen.

Grateful acknowledgment is hereby extended to Dr. G. T. NIGHTINGALE and to Dr. W. C. RUSSELL for many valuable suggestions offered during the progress of this work.

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CARBON DIOXIDE BALANCE AT HIGH LIGHT INTENSITIES

ELMER S. MILLER AND G. O. BURR

(WITH TWELVE FIGURES)

Introduction

In this first paper of a proposed series, it is shown that at a high light intensity all plants studied reached the same limiting CO_2 concentration and maintained this level for many hours of continuous illumination. Ten species of widely different types of leafy plants have been used, and at low temperatures these behaved alike. It appears, therefore, that there is an extremely narrow range of CO_2 concentration which so greatly limits the rate of photosynthesis that differences in rates of respiration and other internal factors have little effect on this point of balance between respired and assimilated CO_2.

Over a wide range this CO_2 balance is independent of temperature. At very high temperatures (36° C.), however, some species no longer maintain the usual CO_2 balance, but reach a much higher value (figs. 6, 8). This is especially emphasized here because of its relation to the abnormal photosynthetic behavior of plants reported recently by KOSTYTSCHEW and coworkers (**8**, **9**).

Apparatus

Although much work has been done with land plants or single leaves, many workers in recent years have turned to the submerged plant because of the ease with which its conditions can be controlled. It is true that in photosynthetic work with high light intensities, leaves or whole plants are subjected to abnormal conditions when inclosed in a glass vessel through which a very slow stream of air is drawn. This makes for abnormally high internal temperatures and humidities because of restricted air cooling and evaporation.

The important feature of the apparatus used for this work is the closed system which permits a continuous and vigorous circulation of air about the plant (fig. 1). This makes possible an easy control of the humidity, temperature, and evaporating power of the air. The leaves may be raised to a high temperature while the roots are kept cool, thus maintaining the plant in an environment which is not unusual. The simple apparatus pictured in figure 1 makes possible a wide range of temperature and humidity. The air temperature can easily be held constant to within ± 1° C. over a period of 12 hours or more without attention. In this work the temperature has been varied from 4° to 37° C. and there is no reason why a still wider

range cannot be just as easily attained. Atmometer readings and transpiration records indicate that the evaporating power of the air has been kept fairly high. The only means of lowering humidity in these experiments has been the condensation of water in the cooled parts of the apparatus. A further reduction of humidity can be accomplished by the insertion of a drying agent in the circulating air stream. Work along this line is being undertaken.

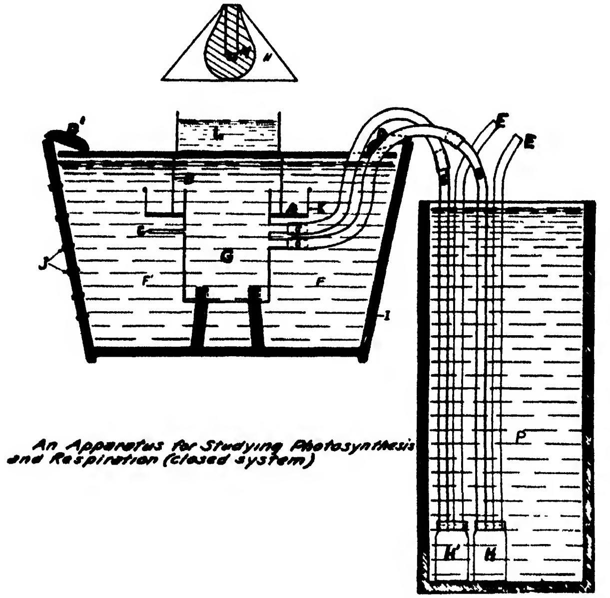


FIG. 1. Photosynthesis chamber and connections.

The plant chamber (fig. 1 *G*) is constructed of tinned copper. This permits ready temperature control in conjunction with the copper tubes immersed in the ice bath (*P*). The pump circulates 10–12 liters of air per minute.

Method of analysis

The carbon and oxygen were determined with the HALDANE-CARPENTER (4) apparatus. All analyses were made in a sub-basement where the experiments were conducted, a place that is only slightly affected by outdoor temperature fluctuations. After preliminary and outside air analyses had been made to check the apparatus, the sample was drawn directly from the chamber through a capillary tube. After washing back and forth five times the sample was taken. Employing this technique, two duplicate determinations can be made to check within the experimental error involved in the manipulation of the machine. After each set of analyses, the capil-

lary tube was opened to the room air for approximately one second to permit an equilibrium with the gases in the plant chamber. Each analysis requires 40 cc. of air; therefore 40 cc. of outside air containing approximately 0.013 cc. of CO_2 were introduced after each analysis. This is negligible.

In this apparatus with a total volume of 8.33 liters for plant chamber, coils, and pump, a gain or loss of 1 cc. of either carbon dioxide or oxygen causes a 0.012 per cent. change in the concentration of the respective gases. For carbon dioxide, the HALDANE-CARPENTER apparatus has an accuracy of $\pm$ 0.001 per cent. or $\pm$ 0.085 cc. of this gas taken up or given off by the plant. For oxygen it has about one-third this accuracy.

In some tests on rates of respiration (experiments 107, 111, and 112, table II), only 13 to 20 mg. of carbon dioxide were evolved; therefore the maximum carbon dioxide error is 2.3 per cent. in studies of these respiratory rates. The percentage error can be greatly reduced by continuing the experiment over a longer period.

Material

For this investigation, potted greenhouse plants of a size that would conveniently fit into the plant chamber were used. The types of plants used were one hydrophyte, seven mesophytes, and two xerophytes. The purpose of using such a variety was to ascertain whether plants with leaves of different thicknesses and characteristic of different habitats responded to conditions in a similar manner. The plants were brought from the greenhouse to the laboratory two hours preceding the experiment. During this period the plants were kept in almost total darkness and at a temperature of approximately 20° C. At the beginning of the experiment, the plants were watered with 30 cc. of water. At the end of each experiment the widths and the lengths of the leaves were measured. From these data from a special set of graphs designed for each species the respective leaf areas were calculated.

Before the use of potted plants was decided upon, a number of experiments were conducted on cut *Pelargonium* stems with adventitious roots 4 to 9 cm. long. These plants were kept in a Shive's nutrient solution (one-third usual concentration). Particular pains were taken to note the temperature of this culture solution at the end of each experiment, and in no case did it exceed 18° C. when the air about the leaves was 35°–37°. Hence even at the higher temperature one was sure that the roots did not suffer from overheating.

When these experiments at 4°–6° and 35°–37° C. were completed, potted *Pelargonium* plants were used and it was found that the curves for the latter were similar to those obtained on the cut stems. It was therefore

concluded that the soil bacteria and the plant roots themselves introduced no appreciable error. It was felt that for metabolism studies extending over many hours, potted plants rather than cut stems would give results more characteristic of the species in its native habitat.

Procedure for CO_2 steady states

The level and temperature of the water were adjusted according to previous experience to give the desired temperature about the leaves. The air in the room was renewed with a large ventilator so that the CO_2 content was less than 0.035 per cent., and the apparatus was filled with this air by running the pump with the chamber lid off. The plant to be studied was then put inside, the chamber closed, the pump started, and the air brought to temperature while the plant was in darkness. The first air sample was drawn for analysis and the light turned on. Since the apparatus was being used as a closed system, rise or fall in percentage of CO_2 and O_2 was a measure of rate of gas exchange, volume being known (0.012 per cent. change = 1 cc. gas).

In experiments 1–10 inclusive the light from a 1000-watt mazda lamp was filtered through 5 cm. of water. The intensity inside the plant chamber was 2050 foot candles (measured with a MacBeth illuminometer). All plants chosen had their leaves spread in a horizontal plane so that they did not shade each other appreciably, and they were approximately the same distance from the light. Each curve shown in figures 2–11 came from a different individual plant. Experiments 1a, 1b, 1g, and 1h were conducted with the cut *Pelargonium*; the others with potted plants.

Duplicate analyses were usually made for each point on the curves.

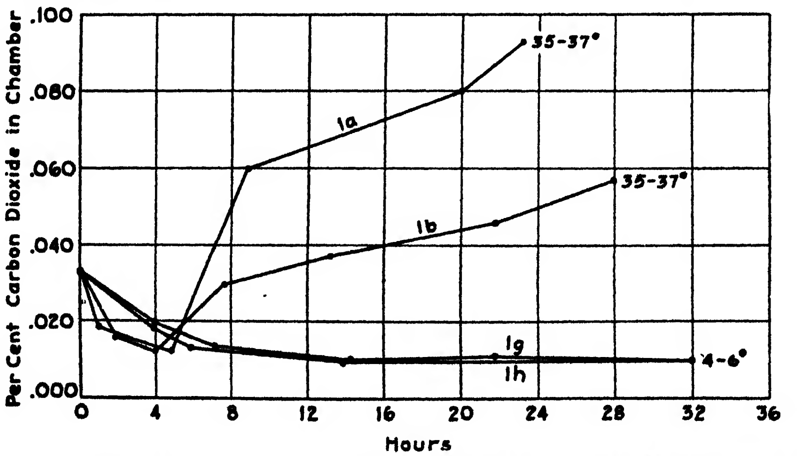


FIG. 2. Effect of temperature on CO_2 exchange of cut stems of *Pelargonium hortorum* Bailey. Light intensity = 2050 f.c.

Results

The leaf area, transpiration, and atmometer values for the 34 plants used in experiments 1–10 are given in table I.

Figures 2–12 show the course of CO_2 exchange following exposure of the plants to 2050 f.c. light intensity. Every plant at some temperature quickly reduces the CO_2 concentration in the chamber to 0.01 volume per cent., a level which is maintained over long periods of time (24 hours or more). No

TABLE I

AMOUNTS OF EVAPORATION AND TRANSPIRATION OF THE PLANTS USED IN EXPERIMENTS 1A TO 10B INCLUSIVE AT DIFFERENT TEMPERATURES

EXPERIMENT NO.	TEMPERATURE	TRANSPIRATION	DURATION	LEAF AREA	EVAPORATION FROM ATMOMETER	TRANSPIRATION/100 CM.² LEAF AREA/24 HOURS
	° C.	gm.	hrs.	cm.²	gm.	gm.
			Pelargonium hortorum			
1a	35–37	17.0	23.0	388		4.57
1b	35–37	21.5	28.0	260		7.09
1c	35–37	47.5	26.0	356	64.80	12.32
1d	35–37	40.0	26.0	237	63.52	15.58
1e	4–6	7.0	32.0	202	14.16	2.60
1f	4–6	7.8	32.0	253	13.60	2.31
1g	4–6	12.7	32.0	406		2.35
1h	4–6	14.5	32.0	348		4.17
			Tolmiea menziesii			
2c	35–37	125.5	38.5	471	46.88	16.61
2d	35–37	128.7	33.0	317	43.52	29.34
2a	22–23	73.8	36.0	378	29.28	13.02
2b	22–23	51.7	24.0	349	26.96	14.81
			Coleus blumei var. *verschaffeltii*			
3a	35–37	33.0	21.25	347	14.40	10.74
3b	35–37	59.5	23.0	321	16.20	19.34
			Bryophyllum pinnatum			
4a	35–37	33.6	15.8	146	17.76	34.96
4b	35–37	56.0	20.8	272	20.16	23.75
4c	6–8	7.5	24.0	170	12.48	4.41
4d	6–8	8.7	25.0	222	12.96	3.76
			Eichhornia crassipes			
5a	35–37	330.0	65.3	182	....	66.63
5b	35–37	155.0	38.0	161	....	58.84

TABLE I (*Continued*)

Experiment No.	Temperature	Transpiration	Duration	Leaf area	Evaporation from atmometer	Transpiration/100 cm.[2] leaf area/24 hours
	° *C.*	*gm.*	*hrs.*	*cm.*[2]	*gm.*	*gm.*
	Crassulaceae sp.*					
6a	35–37	92.3	69.0	234		13.72
6b	35–37	62.5	47.0	295		10.82
6c	4–6	8.8	24.0	234		3.76
6d	4–6	9.0	24.0	295		3.05
	Primula auricula					
7a	35–37	22.3	24.0	567	29.20	3.93
7b	35–37	49.3	23.5	530	28.96	9.50
	Saxifraga sarmentosa					
8a	35–37	74.3	21.5	574	29.20	13.22
8b	35–37	29.4	22.0	386	32.0	7.54
	Zebrina pendula					
9a	35–37	29.2	23.0	297		10.26
9b	35–37	40.0	23.0	548		7.62
9c	16–18	22.0	22.0	297		7.53
9d	16–18	24.2	23.5	548		4.51
	Begonia tuberhybrida					
10a	35–37	54.5	22.5	199		29.21
10b	35–37	48.5	22.5	613		8.44

* It is regretted that, since no fruits of this plant were available, the taxonomists at Minnesota were unable to identify it further for us.

CO_2 is being introduced into the chamber from the outside; it is a closed system in which a horizontal curve indicates that CO_2 respired equals CO_2 assimilated. The slope of a rising curve gives the rate of respiration in excess of photosynthesis and of a falling curve the excess photosynthesis.

The advantage of studying CO_2 balance rather than rates of CO_2 assimilation is forcibly shown by an examination of the curves in figures 2–12. The assimilation rates, as shown by the downward slopes of the curves after the start of the experiment, vary considerably among individuals of the same species and among species. When the total CO_2 assimilated per hour is divided by the leaf area (table I), values ranging from 0.42 to 1.32 cc. CO_2/100 cm.^{2}/hr. are found. Yet these plants all come to the same point of balance.

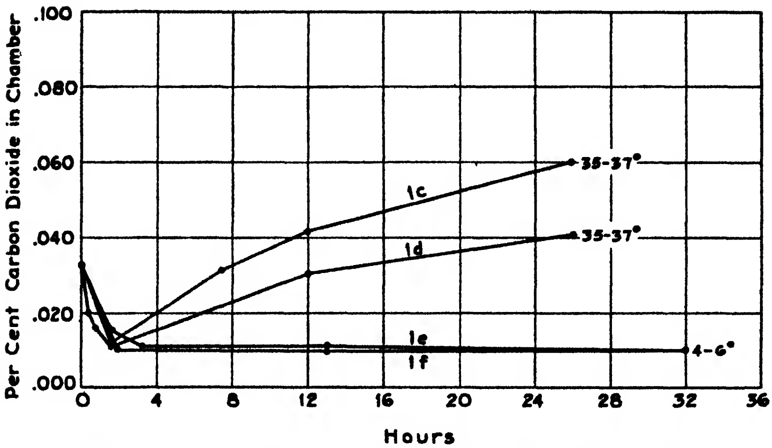


FIG. 3. Effects of temperature on CO_2 exchange of potted *Pelargonium* plants. Light intensity = 2050 f.c.

The reason for this irregularity in rate of assimilation seems clear. Rate is dependent partly upon CO_2 diffusion from air to chloroplast which in turn is regulated by stomal numbers and degree of opening, arrangement of cells and air spaces within the leaf, viscosity of solution within the cell, arrangement of chloroplasts, and other internal factors. MASKELL (11) has given a detailed discussion of internal resistances.

The following points are of special interest:

1. The curves break fairly sharply as 0.010 per cent. CO_2 is approached.
2. The limiting percentage of CO_2 is independent of temperature provided the temperature is not high enough to cause abnormal metabolism (fig. 6).

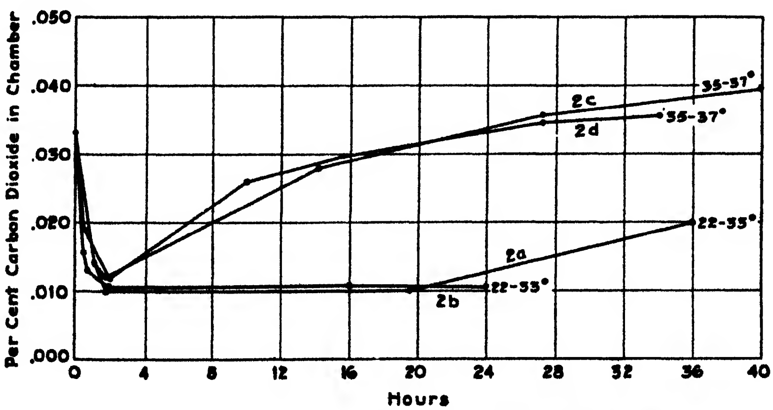


FIG. 4. Effect of temperature on CO_2 exchange of *Tolmiea menziesii* T. & G. Light intensity = 2050 f.c.

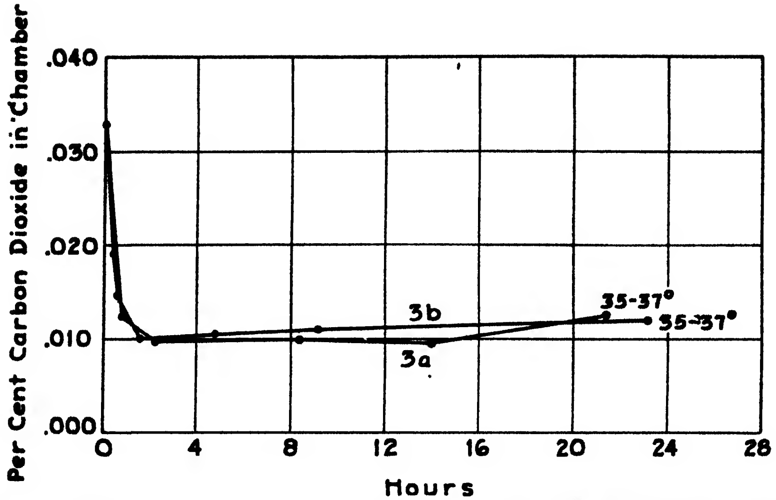


FIG. 5. Effect of temperature on CO_2 exchange of *Coleus blumei* Benth. var. *verschaffeltii* Lem. Light intensity = 2050 f.c.

3. The limiting percentage of CO_2 is independent of leaf properties (thickness, chlorophyll content, sap constants).

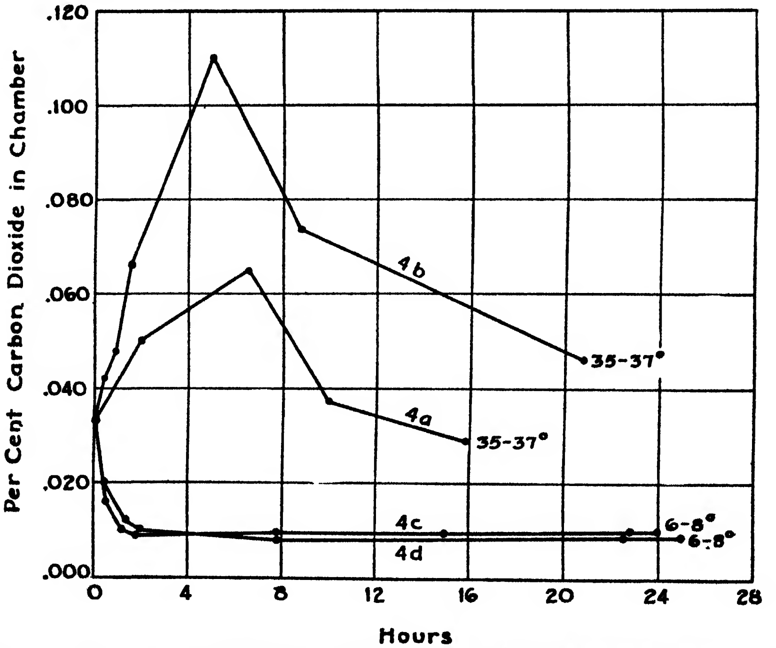


FIG. 6. Effect of temperature on CO_2 exchange of *Bryophyllum pinnatum* Kurz. Light intensity = 2050 f.c.

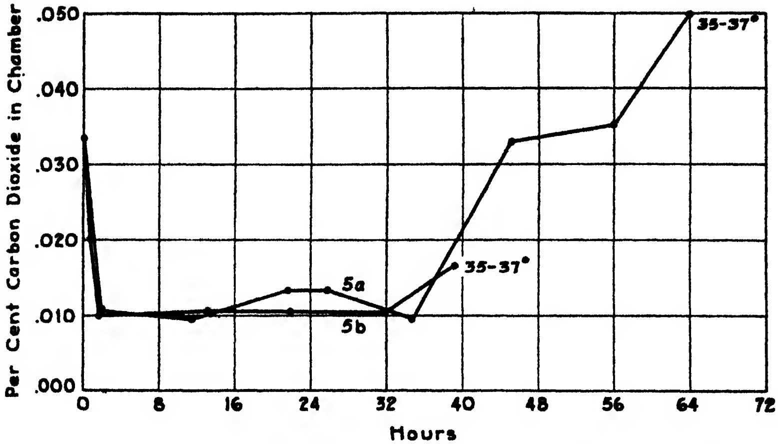


FIG. 7. Effect of temperature on CO_2 exchange of *Eichhornia crassipes* Solms. Light intensity = 2050 f.c.

4. Balance in CO_2 exchange does not necessitate a balance in O_2 exchange.

5. Plants may be segregated into definite groups according to their behavior at high temperatures.

1. The sharp breaks of many of the rate curves as the CO_2 concentration approaches 0.01 per cent. are excellent examples of close approximation to BLACKMAN's (**2**) diagrammatic picture of limiting factors. The available CO_2 in the closed system is quickly consumed at a high rate of apparent assimilation (assimilation in excess of respiration) until the region of CO_2 limitation is reached, at which apparent assimilation becomes zero (respira-

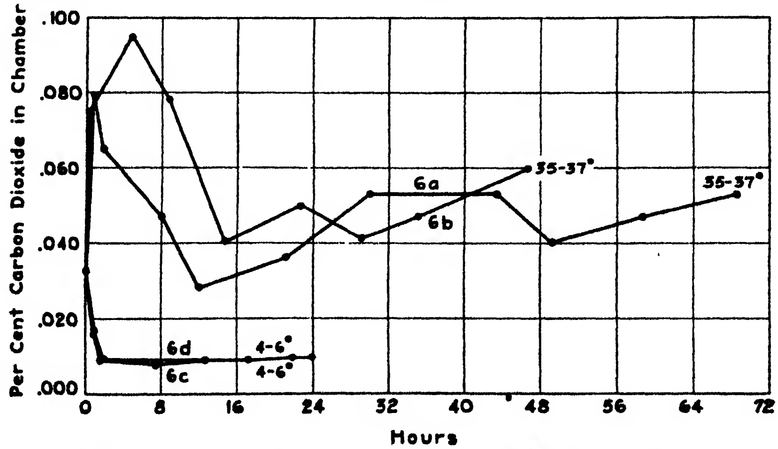


FIG. 8. Effect of temperature on CO_2 exchange of Crassulaceae sp. Light intensity = 2050 f.c.

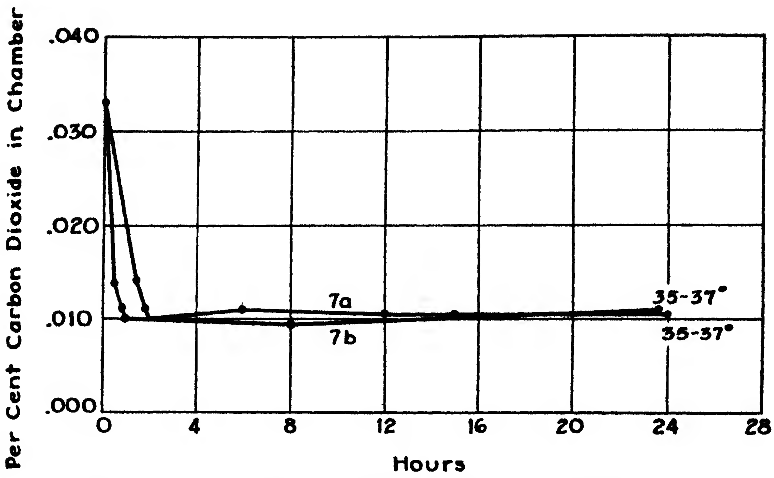


FIG. 9. Effect of temperature on CO_2 exchange of *Primula auricula* L. Light intensity = 2050 f.c.

tion = photosynthesis). These findings are in almost perfect agreement with two of VAN DEN HONERT's (6) curves, which show that at 0.010 volume per cent. CO_2 is the chief limiting factor while at 0.015 volume per cent. it no longer exerts any great influence on the rate of assimilation.

The transition zone is very narrow. It is this narrowness which may partially account for the apparently uniform behavior of all plants studied. Much of the change in slope of the rate curve may take place in a range of 0.005 per cent. CO_2. And if, after reaching 0.010 per cent. CO_2, we con-

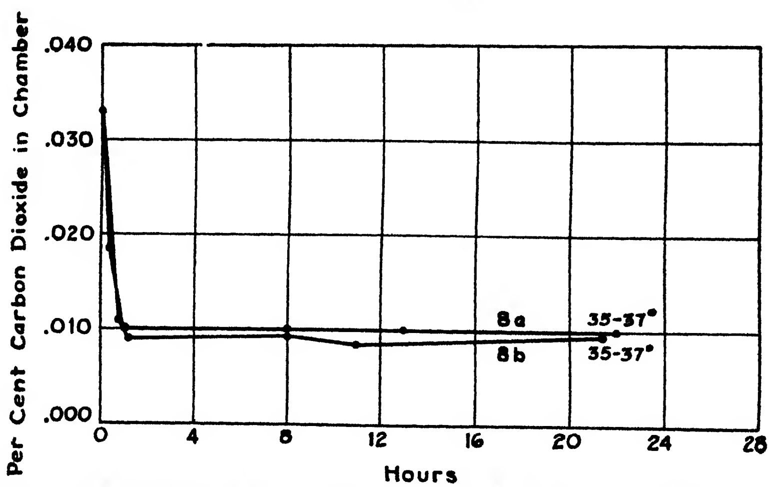


FIG. 10. Effect of temperature on CO_2 exchange of *Saxifraga sarmentosa* L. Light intensity = 2050 f.c.

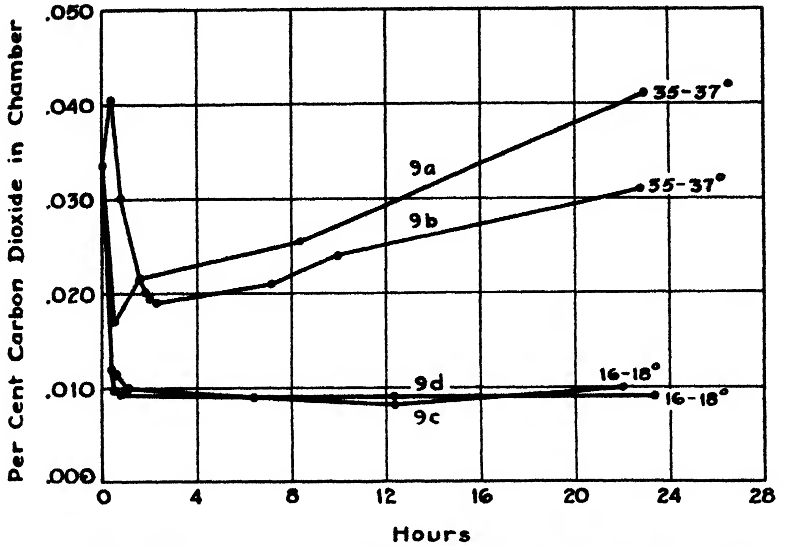


FIG. 11. Effect of temperature on CO_2 exchange of *Zebrina pendula* Schnizl. Light intensity = 2050 f.c.

sider the rate of assimilation directly proportional to CO_2 concentration, a change of 0.002 per cent. will change the rate 20 per cent. Since the minimum error of our analyses is ± 0.001 per cent., it is evident that very appreciable differences might exist between the plants without our detecting them.

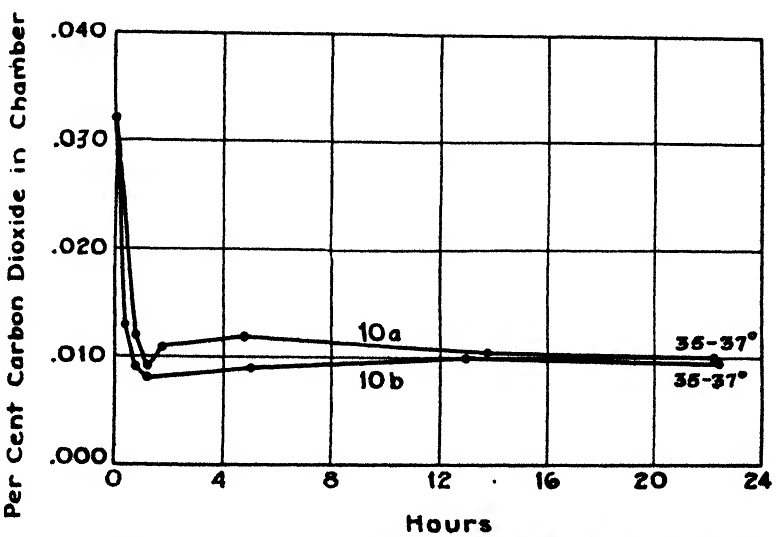


FIG. 12. Effect of temperature on CO_2 exchange of *Begonia tuberhybrida* Voss. Light intensity = 2050 f.c.

It is well known, however, that different plants vary widely in their absolute respiration rates. The rates for some of our plants are given in table II. One *Primula* plant respired at more than twice the rate of one *Pelargonium.* Yet both reach the same CO_2 balance. This suggests that there is a close relationship between ability for respiration and for assimilation, an idea which is not new.

SPOEHR and McGEE (18) state: "It now seems highly probable that a solution of the problem of the internal factor in photosynthesis can be found in the intimate interrelation between photosynthesis and respiration" (p. 76).

WARBURG (22) concluded that the intermediates of respiration might be reutilized in photosynthesis more readily than carbon dioxide.

VAN DER PAAUW (13) concluded that there is a close parallelism between photosynthesis and respiration. Mild stimulation or retardation of both processes can be accomplished by KCN. This does not agree with earlier work of WARBURG (22). ARNOLD (1), however, found that "an unidentified unit in the mechanism of photosynthesis of *Chlorella pyrenoidosa* is rendered inactive by absorption of one quantum of ultra-violet light (2537 Å wave length)" without an appreciable effect on the normal respiration.

0.01 per cent. CO_2 does not represent the limit to which a cell may reduce its CO_2 tension by photosynthesis. The curves here given are for whole potted plants with leaves of varying thickness. Cells on the lower side of thick leaves are in very dim light and their respired CO_2 must be utilized by the lighted cells. There is also the CO_2 from stem, roots, and soil to be taken into account. Therefore 0.01 per cent. may be considered the maximum value to which a whole plant can raise the CO_2 in a closed system when its leaves are in light of 2000 f.c. intensity.

2. It has been found that the CO_2 balance is not altered by considerable changes in temperature. A plant may be kept at 5° or at 36° C. and in both cases reduce the CO_2 to 0.01 per cent. Since at this point respiration equals photosynthesis, the rates of both must increase equally. It is shown in table II that respiration rate in the dark increases about fourteen or fifteen times for both *Primula* and *Begonia.* These species both maintain the 0.01 per cent. level of CO_2 at 36° as well as at 5° (figs. 9, 12). Assuming the same temperature coefficient for respiration in the light, we must conclude that the rate of photosynthesis likewise increased about fifteen times over this same range in temperature. As these steady states were maintained for more than 20 hours, it is evident that the two rates were always identical, for the slightest difference would soon raise or lower the CO_2 concentration a measurable amount.

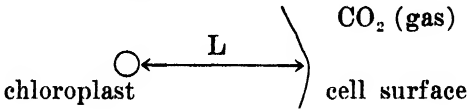
TABLE II

RESPIRATORY QUOTIENTS AND RATES OF RESPIRATION

EXPERIMENT NO.	PLANT	TEMPERATURE	CO_2 RESPIRED	LEAF AREA	RESPIRATORY QUOTIENT	CO_2 RESPIRED/M^2 LEAF AREA/HOUR	RATE, 35°–37° / RATE, 4°–6°
		°C.	%	cm.²		mg.	
101	Pelargonium	35–37	0.945	200	0.85	735.35	57
107		4–6	0.036	300		12.79	
102		35–37	1.361	297	0.80	515.79	
108		4–6		297			
103	Primula	35–37	0.362	397	0.78	265.92	14
109		4–6	0.082	397		19.54	
104		35–37	0.969	376	0.80	421.54	15
110		4–6	0.073	376		28.14	
105	Begonia	35–37	0.806	560	0.79	243.91	15
111		4–6	0.100	560		16.12	
106		35–37	0.551	298	0.78	263.18	14
112		4–6	0.062	298		18.47	

This is further evidence that light is in excess, and that in assimilation, the rate determining step is not the primary process involving light absorption but an intermediate dark reaction limited by CO_2 concentration. Probably the temperature coefficient of this reaction is very close to that of respiration and it may be identical with it. This view is in accord with the recent findings of VAN DER PAAUW (**13**, p. 564), by whom the Q_{10}'s for photosynthesis and respiration were shown to be almost identical over the range 10°–30° C. The experimental method employed by investigators of CO_2 balance is especially good because it eliminates much of the diffusion resistance and permits the comparison of photosynthesis and respiration in the same leaf at the same time.

A real difficulty arises when a mechanical picture of the utilization of respired CO_2 is considered. The following diagram illustrates the relation between CO_2 and the chloroplast within the cell:



CO_2 (gas) is in equilibrium with CO_2 (dissolved) at the surface of the cell. Let C_1 be the concentration of CO_2 (dissolved) at the cell surface and C_2 the concentration at the chloroplast surface. Then the rate of diffusion of respired CO_2 from surface protoplasm to chloroplast surface is proportional to $\frac{C_1 - C_2}{L}$, where L is the distance. When the temperature was raised 31° the respiration in the dark increased about fifteen times. In order that the respired CO_2 at the cytoplasm surface reach the chloroplast fifteen times as fast, the gradient $\frac{C_1 - C_2}{L}$ must increase fifteen times[1] The surface concentration, C_1, does not increase since there is no rise in the CO_2 (gas). If the distance, L, remain constant then the only way to increase the gradient would be by a drop in C_2. An appreciable drop in CO_2 does not seem likely since assimilation shows the expected increase in rate with temperature rise.

The simplest explanation of the utilization of the increased products of respiration without an increase in CO_2 tension seems to be that an intermediate product of respiration is reutilized before any CO_2 is liberated. This agrees with the suggestion, previously stated, that there is a very

[1] VAN DEN HONERT (6) has pointed out that fall in viscosity is about equal to the fall in solubility of CO_2 with rise in temperature. Therefore, when the diffusion of CO_2 is the chief limiting factor in assimilation a Q_{10} of 1 is to be expected. His experimental data support this view. Hence the diffusion factor is eliminated from our consideration.

close relationship between photosynthesis and respiration, and is in line with the suggestion of WARBURG (22) that possibly an intermediate product of respiration is utilized in photosynthesis when no CO_2 is being exchanged by the plant.

3. The balance between respiration and photosynthesis is reached at a definite CO_2 tension. No measurements of pH were made on the leaves studied, but it is highly improbable that all ten species had the same hydrogen ion concentration at the time of the experiment. Without assuming any definite pK'_1 for the cell saps, on rearranging the Henderson-Hasselback equation to

$$\log \frac{\text{combined } CO_2}{\text{dissolved } CO_2} = pH - pK'_1$$

it becomes clear that there is a fixed ratio of combined to dissolved CO_2 when $pH - pK'_1$ is a constant. It is not likely that all plants used in this investigation assumed such a fixed relation between pH and pK'_1. When $pH - pK'_1 = -0.3$ dissolved CO_2 is twice combined CO_2; when $pH - pK'_1 = +0.3$ combined CO_2 is twice dissolved CO_2.

If these assumptions are correct, at constant CO_2 tension the different plants differed widely in combined CO_2, and it may be concluded that the steady state observed is a function of dissolved CO_2 and that the rate of photosynthesis is limited by the concentration of H_2CO_3 and not by bicarbonates.

This view is in accord with the theory of WILLSTÄTTER and STOLL (23, p. 244) for the combining of H_2CO_3 with chlorophyll. It is probable that $NaHCO_3$ could not enter into this reaction. JAMES (7), VAN DEN HONERT (6), and others have assumed that only dissolved CO_2 is available for assimilation.

4. In experiments 101–106 (table II) the total gas exchange is great so that the respiratory quotients are of high accuracy. Their values range from 0.78 to 0.85. These are in agreement with the values found by MAQUENNE and DEMOUSSY (10) for young leaves after a few hours in darkness. Our plants were in the chamber several hours at the high temperatures, hence the low respiratory quotients are to be expected.

If we accept the value of 1.00 for photosynthetic quotient, it is evident that when CO_2 is in balance O_2 will be disappearing, *i.e.*, the plants are not in energy equilibrium. These respiration experiments were continued through a period of CO_2 equilibrium. While the change in CO_2 concentration was zero, the O_2 changed appreciably (table III).

This brings up an interesting question in regard to compensation point. PLAETZER (14) defined compensation point as that light intensity at which gas exchange in a leaf is zero. Neither gas was specified. PLAETZER and

TABLE III

CHANGE IN CONCENTRATION OF OXYGEN AND CARBON DIOXIDE AFTER CO_2 EQUILIBRIUM HAD BEEN MAINTAINED SEVERAL HOURS

EXPERIMENT NO.	PLANT	TEMPERATURE	DURATION	CHANGE IN CO_2 CONCENTRATION	CHANGE IN O_2 CONCENTRATION IN 5 HOURS
		° C.	hr.	%	%
101	*Pelargonium*	35–37	5.071	+ 0.002	– 0.105
102	*Pelargonium*	"	6.071	– 0.001	– 0.077
103	*Primula*	"	6.000	+ 0.002	– 0.079
104	*Primula*	"	5.500	± 0.000	– 0.087
105	*Begonia*	"	4.500	± 0.000	– 0.077
106	*Begonia*	"	4.500	+ 0.001	– 0.084
107	*Pelargonium*	4–6	5.750	± 0.000	– 0.012
108	*Pelargonium*	"	4.750	+ 0.001	– 0.053
109	*Primula*	"	4.500	± 0.000	....
110	*Primula*	"	8.000	– 0.001	– 0.053
111	*Begonia*	"	5.000	– 0.001	– 0.053
112	*Begonia*	"	4.500	± 0.000	– 0.038

HARDER (**5**) have used O_2 exchange as the measure, while BOYSEN-JENSEN (**3**), MÜLLER (**12**), and others follow CO_2 exchange. Since we have reason to believe that O_2 consumption and CO_2 evolution in respiration are distinct reactions with different characteristics (**19–21**), it would be well to specify which gas is to be in balance at the compensation point.

5. An examination of figures 2–11 shows that plants exhibit a great variability in their behavior at 35°–37° C. They may be classified into three general groups: (1) Those which are stable at 35°–37° and reach the same point of CO_2 equilibrium as shown at lower temperatures (*Coleus, Eichhornia, Primula, Saxifraga, Begonia*); (2) those which temporarily follow the low temperature curve and then begin active CO_2 evolution (*Pelargonium, Tolmiea, Zebrina*); (3) those which evolve large quantities of CO_2 as soon as they are raised to 35°–37° and do not in the course of the run reduce the CO_2 concentration below that of normal air (*Bryophyllum* and Crassulaceae sp.).

These curves show the danger in working at such high temperatures when studying CO_2 assimilation, and probably explain some of the recent results of KOSTYTSCHEW and co-workers (**8, 9**), who have carried out extensive investigations of the daily course of photosynthesis of plants of central Asia. They inclosed the attached leaf in a glass chamber in the field and with an aspirator drew a small volume (about 11 liters) of air across the leaf. Most of their runs did not exceed 30 minutes. The temperature inside the chamber was not recorded, but the outside temperature was often

TABLE IV

CHANGES IN CONCENTRATION OF O_2 AND CO_2 IN PLANT CHAMBER DURING PERIOD OF MAXIMUM GASEOUS EVOLUTION

EXPERIMENT NO.	PLANT	TIME AFTER START OF EXPERIMENT							
		3 HOURS		4 HOURS		5 HOURS		6 HOURS	
		CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
		%	%	%	%	%	%	%	%
4a	*Bryophyllum*	+0.019	+0.082	+0.013	+0.071				
4b	*Bryophyllum*	+0.057	+0.017	+0.055	+0.020				
6a	Crassulaceae sp.	+0.032	+0.070	+0.025	+0.068				
6b	Crassulaceae sp.					+0.056	+0.056	+0.054	+0.040

as high as 37°. In bright light there was undoubtedly a considerable rise of leaf temperature within the chamber. KOSTYTSCHEW and his colleagues observed sharp fluctuation in photosynthesis, especially by the xerophytes in the Bogar plots. There were short flashes of photosynthesis alternated with short but energetic evolution of carbon dioxide in the light.

It is evident from figures 2–11 that at high temperatures almost any kind of value for CO_2 assimilation might be obtained with the different species, and that a 30-minute run made immediately after raising the temperature does not give a true picture of CO_2 exchange. For example, during the first two hours *Pelargonium* shows a high rate of photosynthesis and Crassulaceae sp. a high rate of CO_2 evolution, but at the eighth hour *Pelargonium* is evolving CO_2 and Crassulaceae sp. is carrying on rapid photosynthesis. At lower temperatures, however, all plants show uniform photosynthesis curves.

There are at least two kinds of CO_2 evolution shown in figures 2–11. That which is evolved immediately (figs. 6, 8) must be present in the leaf as dissolved and combined CO_2 (including carboxylic acids) which is released by the rise in temperature and high light intensity. That CO_2 which begins coming off only after several hours at the higher temperature (figs. 2, 3, 4, 11) probably represents CO_2 of metabolism after the high temperature has caused metabolic readjustments within the leaf.

In order to show that the carbon dioxide which came from *Bryophyllum* and Crassulaceae sp. was not produced by respiration suddenly speeded up, those runs were repeated and both oxygen and carbon dioxide were determined. The results are given in table IV. Since both gases showed marked increment, the increase in CO_2 cannot be attributed to normal aerobic respiration.

One hundred cc. of distilled water dissolves from air at 760 mm. and 20° C. 0.026 cc. CO_2 and 0.649 cc. O_2; at 35° C. 0.018 cc. CO_2 and 0.501 cc. O_2. Assuming the same change in solubility in leaf sap, a rise of 15° C. would liberate only 0.148 cc. O_2 from 100 cc. of leaf tissue, so that the

TABLE V

EXPERIMENT NO.	PLANT	CO_2 EVOLVED PER GM. DRY LEAF
4a	*Bryophyllum*	4.64
4b	*Bryophyllum*	5.06
6a	Crassulaceae sp.	3.97
6b	Crassulaceae sp.	2.33

solubility factor is negligible. The oxygen most likely comes from photosynthesis and table IV indicates that O_2 increases at the expense of CO_2 (experiments 4a and 6a). Therefore the total CO_2 evolved would really be equal to $CO_2 + O_2$.

Table V gives the calculated CO_2 liberated by the leaf material at the time of greatest $CO_2 + O_2$ increase measured.

These are much larger values than found by SPOEHR and MCGEE (**18**) for the total CO_2 combining power of dried leaves, excepting sunflower (13.9) and nettle (6.2). The release of all combined (bicarbonate) CO_2 requires a large increase in acidity (pH 4.5) which probably does not take place. It seems likely, therefore, that much of the CO_2 liberated at high temperatures arises through a fermentation type of respiration, including decarboxylation.

It should be pointed out that when plants are in CO_2 equilibrium with the air, there is little possibility of their holding very considerable quantities of either free or combined CO_2 (as bicarbonates). In the Henderson-Hasselback equation

$$\mathrm{pH} = \mathrm{pK}_1' + \log \frac{\text{combined } CO_2}{\text{dissolved } CO_2}$$

no definite value can be set for pK_1'. Its value for sea water is given by SAUNDERS (**16**) as 6.06 and the accepted value for blood is 6.1. If a value of 6.0 is assigned to the average plant, then at pH 6.0 the volume of combined CO_2 would be equal to dissolved CO_2. Recorded values indicate that most plant saps are rarely more alkaline than pH 6. Therefore with a maximum possible CO_2 solubility at 20° C. of 0.026 cc. per 100 cc. of leaf sap, the total CO_2 in the leaf could rarely be more than 0.05 cc. per 100 cc. of plant sap. This limiting value would not hold if gaseous CO_2 were trapped within the leaf at a concentration above that found in air.

RICHARDS (**15**) observed very high rates of CO_2 evolution when joints of cactus (*Opuntia versicolor*) were exposed to light. In diffuse light (intensity not stated) the rate of CO_2 evolution was about the same as in the dark, but very little O_2 was absorbed so that the respiratory quotient rose to values as high as 7.00. In direct sunlight, CO_2 was evolved at a somewhat lower rate and O_2 came off at the same time. RICHARDS likewise observed that when there was high CO_2 evolution there was low O_2 evolution. The results of our work at high temperatures with *Bryophyllum* and Crassulaceae sp. described in this work are in close agreement with the work of RICHARDS. He attributes the high CO_2 production in light to the photochemical decomposition of organic acids, and shows a large decrease in total acidity to support this view. The runs in which both O_2 and CO_2 egressed were made at high temperatures (30°–39° C.), and RICHARDS

realized that this was probably an important factor. The normal photosynthetic behavior of *Bryophyllum* and Crassulaceae sp. at 4°–6° C. substantiates his suggestion that *Opuntia* stores energy only in cooler seasons, while it grows at the expense of this stored food in hot weather. SPOEHR (17) has further discussed the photolysis of organic acids in the cactus.

In view of the results here reported, it must be concluded that temperature is fully as important as light in determining the rate of evolution of CO_2 from plants exposed to light. In fact the photosynthesis curves of *Bryophyllum* and Crassulaceae sp. at 4°–6° C. are so similar to the curves of other leaves that it would appear that no decarboxylation takes place in 2000 f.c. light intensity at the low temperature. That temperature is the chief factor causing the evolution of CO_2 by *Pelargonium* is shown by the enormous increase (fifty-seven times) in dark respiration of this species with a 31° rise in temperature (table II, experiments 101 and 107).

KOSTYTSCHEW, *et al.* observed the greatest CO_2 evolution by xerophytes of central Asia. The work of RICHARDS with *Opuntia* and the work here reported, combined with that of KOSTYTSCHEW, would point to a specialized metabolism not merely of succulents but of xerophytic plants generally. The recent work of TANG (19–21) again strongly emphasizes the view that CO_2 evolution and O_2 consumption must be considered as separate processes with different mechanisms and specific characteristics.

Summary

Under the conditions of these experiments the following conclusions may be drawn:

1. Many types of potted plants in a closed system quickly reduce the CO_2 to 0.01 volume per cent. and maintain this concentration in light of about 2000 f.c. intensity.

2. The sharp break in the rate curve indicates a close approximation to BLACKMAN'S ideal graph for limiting factors.

3. CO_2 balance is independent of temperature, suggesting that a dark reaction of photosynthesis has a temperature coefficient equal to the temperature coefficient of respiration.

4. CO_2 balance depends on the concentration of dissolved CO_2 and not on total CO_2 (H_2CO_3 plus bicarbonates).

5. Plants in CO_2 balance are not in an energy balance because the respiratory quotient under many conditions is not unity.

6. Evolution of CO_2 from succulents and xerophytes in bright light is caused by high temperature rather than by light.

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OSMOTIC QUANTITIES OF PLANT CELLS IN GIVEN PHASES[1]

ALFRED URSPRUNG

(WITH TWO FIGURES)

I. Osmotic quantities to be distinguished

Some authors use seven or eight terms, others content themselves with two or even a single one. The points of view are much divided. In order to reach an objective judgment, let us consider a cell from the pith of *Impatiens noli-tangere,* a cell which has been accurately measured by URSPRUNG and BLUM (**48**), MOLZ (**28**), URSPRUNG and BECK (**44**), and URSPRUNG (**46**). Cf. also BECK (**1**). Let V represent the volume of the given cell, and the indices n, g, and s, represent the normal phase, incipient plasmolytic phase (grenzplasmolytischen), and saturation phase respectively. We distinguish (fig. 1, schematic sketch of cell) the normal volume ($V_n = 14{,}122$ units) of the unchanged cell, the volume at incipient plasmolysis ($V_g = 13{,}209$ units), and the volume at complete saturation ($V_s = 14{,}779$ units). V_n had at the time of observation the value just given, which, however, changed as the water balance within the cell changed.

If we desire to measure the osmotic potential of the cell sap of the individual cell, we must begin with the phase of incipient plasmolysis. By means of the plasmolytic method we find first that the incipient plasmolysis value (*i.e.*, the osmotic value at incipient plasmolysis) is $O_g = 0.38$ mol cane sugar. This concentration of the cane sugar solution will cause the protoplasm of our cell to recede from the cell wall ever so little. In the absence of complicating factors, so that the volume changes of the cell have no other effects than corresponding changes in the concentration of the cell sap, one can calculate the osmotic value of the normal sap, O_n, from $O_g = 0.38$ by the equation: $O_n = O_g \frac{V_g}{V_n} = 0.355$ mol cane sugar. If we place the cell sap or an isosmotic cane sugar solution in an osmometer with a semipermeable membrane, the cell sap at incipient plasmolysis would develop an osmotic pressure (physicist's terminology) or a suction force (suction tension, suction[2]) (our terminology[3]) of 10.5 atm. In the condition of equilibrium the protoplasm must have the same suction force. Hence we may write: ***The suction force (suction tension, suction) of the contents of the cell in the phase of incipient plasmolysis*** is $S_{i_g} = 10.5$ atm. Similarly the suction force (suction tension, suction) of the contents of

[1] Translated by WILLIAM A. BECK.

[2] Original: Saugkraft (Saugspannung, Saugung).

[3] The pros and cons of the matter of terminology will be discussed later.

the cell in the normal phase, S_{i_n}, or since for the sake of simplicity the modification referring to the phase is omitted, the *suction force* (*suction tension, suction*) *of the contents of the cell,* $S_{i_n} = 9.7$ atm. In a similar manner it is deduced that $S_{i_s} = 9.3$ atm.

The wall pressure (W), *i.e.*, the pressure which the stretched wall exerts upon the contents of the cell has the following values: At incipient plasmolysis naturally $W_g = 0.0$ atm.; in the saturation phase, $W_s = 9.3$ atm.; with the assumption that change in wall pressure is proportional to the change in volume the wall pressure in the normal phase may be calculated and shown to be $W_n = 5.4$ atm.

The turgor pressure (T), *i.e.*, the pressure which the contents of the cell exert upon the wall, is, at equilibrium, numerically equal to the wall pressure, since action and reaction are equal, but it acts in the opposite direction.

The *suction force* (*suction tension, suction*) *of the cell* (S_z) is the force per unit area with which the whole cell, consisting of the cell wall and the contents of the cell, tends to absorb water. If external forces that tend to produce tension or pressure are absent, it is composed of two forces (tensions) which operate in opposite directions: the suction force of the contents of the cell, and the wall pressure, which tends to force water from the cell. The relations may be expressed by the equation:

$$S_z = S_i - W$$

It follows that the *suction force of the cell,* in the phase of incipient plasmolysis, in the normal phase, and in the phase of saturation, has the following values respectively:

$$S_{g_z} = S_{i_g} - W_g = 10.5 - 0.0 = 10.5 \text{ atm.}$$

$$S_{z_n} = S_{i_n} - W_n = 9.7 - 5.4 = 4.3 \text{ atm.}$$

$$S_{z_s} = S_{i_s} - W_s = 9.3 - 9.3 = 0.0 \text{ atm.}$$

Our suction force equation contains three osmotic quantities which differ in their concepts and usually also in their numerical expression. It appears from the numerical expression given above, and even more clearly from the graphic representation in figure 1, that these three quantities behave in an altogether different manner as the cell changes from the phase of incipient plasmolysis to the phase of saturation. S_i varies but little; W increases very rapidly; and S_z decreases even more rapidly. Let particular emphasis be placed on the fact that the same cell may simultaneously possess an inner pressure of several atmospheres ($T_n = 5.4$ atm.), and nevertheless be able to take in water ($S_{z_n} = 4.3$ atm.).

Previously it was assumed that no foreign mechanical stress or strain was placed upon the cell; in this case T = W numerically. If an external pressure (+ A) or tension (− A) is present, then T = W ± A, and the equation expressing the suction force takes the form: $S_z = S_i - (W \pm A)$.

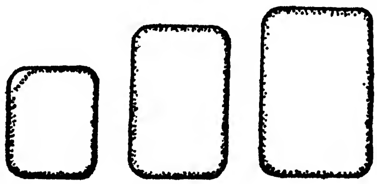
Significance of the osmotic quantities

The significance of the osmotic quantities follows consequently from what has already been said.

The *suction force* (*suction tension, suction*) of the cell, $S_{z_n} = 4.3$ atm., is the quantity that is the dimensional standard for the intake, the extrusion, and the conduction of water. It is indispensable, for example, in the study of water economy.

The *suction force* (*suction tension, suction*) *of the contents of the cell*, $S_{i_n} = 9.7$ atm., must not be confounded with S_{z_n}. Thus it does not deter-

Zustand der Zelle	Grenz-plasm. g	Nor-mal n	Wasser-sätti-gung s
Volumen der Zelle (V) . .	13 209	14 122	14 779
Saugkraft des Zellinhaltes (Si) in Atm.	10,5	9,7	9,3
Wanddruck (W) in Atm.	0.0	5,4	9,3
Saugkraft der Zelle (Sz) in Atm. . . .	10,5	4,3	0,0



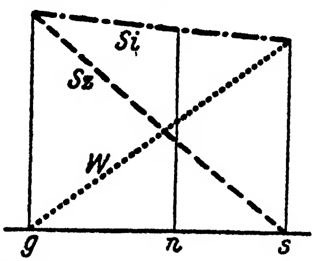


FIG. 1. Graphic representation of the osmotic quantities of a cell taken from the pith of *Impatiens*. (According to URSPRUNG 46.)

mine the amount of water absorption, but it is an important component of S_{z_n}, as is shown by the equation $S_{z_n} = S_{i_n} - W_n$. S_{i_n} is equal to the suction force of the protoplasm. It is furthermore a measure for the change in the water balance of the cell, provided the osmotic solute remains constant.

The *wall pressure,* $W_n = 5.4$ atm., naturally plays an important rôle in turgor movements. The measurement of this quantity has furthermore contributed in establishing the relations that exist between turgor pressure and growth. The rigidity of the soft-walled cell is the result of the combined action of the wall pressure and the turgor pressure. In the state of equilibrium $T = W$; when, however, asci burst open in plasmoptyse $T > W$.

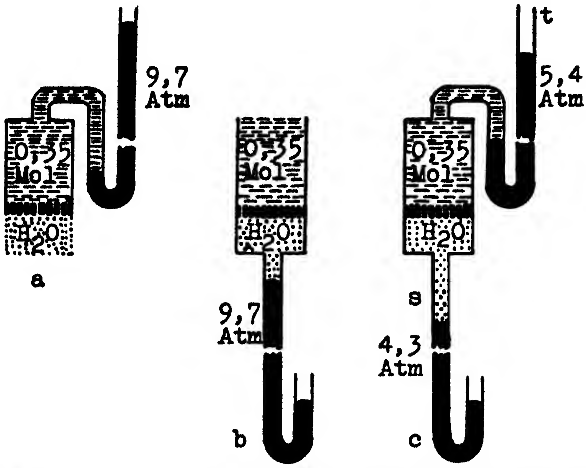


FIG. 2. Diagram of the suction force of an osmometer. (According to URSPRUNG 46.)

Turgor distention. For every change in volume, $V_s - V_g$, there is a corresponding equivalent change in turgor pressure, $T_s - T_g$, which will vary in different cells, the magnitude of which depends upon the distention. The turgor distention is the dimensional standard for the cell, in its rôle as a water reservoir, as also for turgor movements.

The *suction force* (*suction tension, suction*) *at incipient plasmolysis,* $S_{i_g} = S_{z_g} = 10.5$ atm., serves in the study of the regulation of osmotic relations (Studium der Osmoregulationen).

The *osmotic values,* O_g and O_n, are usually auxiliary quantities that are necessary in the determination of S_{i_g} and S_{i_n} of the individual cells.

II. Methods of measuring

Nothing more can be done here than to indicate the principle involved in each of the different methods and thus bring into greater relief the names employed to designate the various quantities.

1. The osmotic value at incipient plasmolysis, $O_g = 0.38$ mol cane sugar, is measured by the oldest method of measuring in this domain. It determines that concentration of a harmless non-permeable plasmolyte which at osmotic equilibrium causes the protoplasm to recede ever so little from the cell wall.

2. The *suction force (suction tension, suction) of the cell or of the cell contents at incipient plasmolysis,* $S_{z_g} = S_{i_g} = 10.5$ atm., is obtained by translating the value 0.38 mol cane sugar, obtained in method 1, into equivalent atmospheres. O_g and S_{i_g} refer, it is true, to an abnormal condition of the cell, but they nevertheless yield important results especially concerning the regulation of the osmotic relations in the normal cells.

3. The *suction force (suction tension, suction) of the contents of the cell in the normal phase,* $S_{i_n} = 9.7$ atm., may for example be determined by cryoscopy of the expressed juice. It must be remembered, however, that all methods employing press-juice yield only average values for the larger tissues or entire organs. The measurement of S_{i_n} for *individual cells* demands a knowledge of the value of O_n.

4. The *osmotic value of the contents of the cell in the normal phase,* $O_n = 0.355$ mol cane sugar, is deduced from O_g by means of the equation $O_n = O_g \frac{V_g}{V_n}$. The equivalent value in atmospheres is the value of S_{i_n}. The value of S_{i_n} cannot be determined directly from S_{i_g} by using the quotient of the volumes as a factor, because the suction force (osmotic pressure) of a solution increases at a greater rate than does the concentration.

5. The *suction force (suction tension, suction) of the cell* in the normal phase, $S_{z_n} = 4.3$ atm., is measured by determining the concentration of a harmless non-permeable plasmolyte (Osmotikum) in which the original cell volume remains constant. It is necessary that the cell wall is sufficiently distensible that the cell may show an appreciable change in volume with change of the external concentration.

6. The *wall pressure,* $W_n = 5.4$ atm., can be calculated for individual cells, in the absence of external pressure, from the equation $W_n = S_{i_n} - S_{z_n}$. The suction force of the cell, S_{z_n}, remains unintelligible so long as the wall pressure is not taken into consideration.

7. The *turgor pressure,* $T_n = 5.4$ atm., is numerically equal to the wall pressure in the absence of external forces, but it acts in the opposite direction. When external forces enter into consideration, then $T_n = W_n + A_n = S_{i_n} - S_{z_n}$.

III. Review and critique of the various terminological proposals

A. The older terminology

Before the newer methods were developed, it was customary to use either the method of incipient plasmolysis or the cryoscopic method. Sup-

pose we begin with the method of incipient plasmolysis, again taking our numerical examples from the data obtained with cells of *Impatiens.* The suction force at incipient plasmolysis was *measured,* $S_{z_g} = S_{i_g} = 10.5$ atm. The values obtained were frequently labeled as follows:

Osmotic pressure; this, however is actually only 9.7 atm.; error = 0.8 atm.

Turgor pressure; actual value is 5.4 atm.; error = 5.1 atm.

Suction force; actual value is 4.3 atm.; error = 6.2 atm.

The confusion is even greater in the following example: the author is studying water intake; he *requires to know* the suction force of the cell (4.3 atm.), but he *speaks* of turgor pressure (5.4 atm.) and he *measures* the suction force at incipient plasmolysis (10.5 atm.).

The difficulties of the cryoscopic method are similar. When this method is used to investigate water economy, *e.g.,* DIXON (**6**), HARRIS and his associates (**14–19**), KORSTIAN (**24**), the quantity determined is not the correct one. For there is no doubt that, in connection with water intake, the suction force of the cell contents does not come into consideration at all, but rather the suction force of the cell (4.3 atm.). The error of all investigations of water economy that depend on the cryoscopic method lies in neglecting to take the wall pressure into consideration.

The misunderstandings that have arisen as the result of the confusion of the osmotic quantities have been discussed elsewhere (URSPRUNG and BLUM **48**); further examples may readily be found by any one in the language with which he is familiar. The task of the new terminology must be to provide a nomenclature such that misconceptions will be avoided.

B. THE RECENT TERMINOLOGY

The quantities which are to be distinguished have been indicated in sections I and II. The individual terms will now be discussed.

INDIVIDUAL TERMS: (1) THE SUCTION FORCE OF THE CELL, $S_{z_n} = 4.3$ ATM.—As previously mentioned, the normal phase is indicated by the omission of the modification referring to the phase; consequently "suction force of the cell" is used instead of "suction force of the cell in normal phase." Of all the terms the expression "suction force" has been most objected to. Some authors criticize only the element "force" (Kraft); others reject the term "suction" as well.

(a) *Pros and cons of "suction."*—SHULL (**36**), to my knowledge the only author who has expressed himself as opposed to the term "suction" (Saugung), says: "If a careful analysis of water movement is made it will always be found that water is the active compound. The cell contents merely provide a medium of lower free water content into which water from regions of greater free water content moves. The pressure in any cell is caused by the free water entering from outside the cell. If the pressure in

a street car, when overcrowded with people who try to enter after the car is full, can be called a suction pressure, then the pressure in a cell can be called suction pressure, and the force of entry called suction tension."[4] In answer to these statements the following remarks may be permitted. So far as I know, the actual cause of the osmotic phenomenon is unknown even today (*cf.*, for example, FINDLAY **9**); consequently there is no necessity for the physiologist to restrict the terminology to any particular theory.

The ordinary mode of expression *favors* the term "suction"; then there is in use a very old expression which we find for example in suction pump, suction root, suction hair. The justification of the term suction appears most clearly if the cell is replaced by a semipermeable osmometer to which in an appropriate manner a manometer has been attached. The osmometer contains a 0.355 mol cane sugar solution (fig. 2, *c*), which has an osmotic pressure of 9.7 atm. The wall pressure, numerically equal to the turgor pressure, is compensated by the mercury pressure of 5.4 atm. in the manometer t. Of special interest to us is the osmotic quantity, which amounts to 4.3 atm. (9.7–5.4 atm.); manometer s (fig. 2) registers it, and clearly as a suction which the osmometer exerts upon the water outside. According to the data indicated on the manometer s, which are objective and independent of any particular concept of the mechanism of osmosis, we have the right to speak of a "suction" force of the cell $S_{zn} = 4.3$ atm.

(b) *Pros and cons of "force."*—SHULL (**37**) says of suction force: "No unsuitable term should be considered immune from change, especially one not over 15 years old, just because it has been commonly employed in Europe." This assertion concerning the age of the contested term does not by any means agree with the facts. Incidentally PFEFFER (**32**, p. 77) speaks of "suction force," but particularly DEVRIES (**51**, p. 561); since that time the term is found in the literature of plant physiology in ever so many languages [*e.g.*, NOLL (**42**) in STRASBURGER's Lehrbuch, p. 161; HABERLANDT (**13**, p. 351); FITTING (**10**, p. 209); VINES (**50**, p. 429); EWART (**8**, p. 77).] Consequently the term "suction force" is about as old as the history of osmotic relations in cells. Since it did not seem to me absolutely necessary to coin a new term, while developing mensural methods in 1916, I employed the term already in use. That the term is not strictly correct in the physical sense, I have already in 1916 emphatically pointed out (URSPRUNG and BLUM **47**, p. 529) as follows: "With reference to the terminology, the term "force" should be retained, even though it is a matter of $\frac{\text{force}}{\text{area}}$, therefore a quantity not mensurable in kilograms, but in atmospheres."

[4] SHULL (**38**) also says in a later paper: "If the expansive force exerted in the interior of an automobile tire when we force air into it can legitimately be called a 'suction force,' then also we can call the expansive force of a cell when water is forcing its way into the cell a 'suction force.' "

(c) *Suggested substitutes for "force."*—Suction "pressure"—as suggested by STILES (**40, 41**), and BRIEGER (**4**), a student with RENNER—has the correct dimensions but is a physical impossibility, since a manometer can register either suction or pressure, but not both at the same time (URSPRUNG **45**, p. 196). The expression appears to have been abandoned, at least in German-speaking countries (RENNER **35**, p. 749; GRADMANN **12**, p. 635).

Suction "value" (GRADMANN **11, 12**, p. 636), suction "ability" (PRINGSHEIM **33**, p. 749), suction "potential" (OPPENHEIMER **30**, p. 131; **31**, p. 526) are terms that indicate sufficiently that there is a question of *capacity* which does not by any means always lead to water intake. This I had already said in 1916 (URSPRUNG and BLUM **47**, p. 530) and still more clearly in 1920 (URSPRUNG and BLUM **48**, p. 202) with the definition: Suction force of the cell = the force with which the cell *strives* to take in water. Of course it is evident that the cell cannot take in water if it does not suck more forcibly than the surrounding medium. I did not deem it necessary to emphasize that fact by applying a special term. One does not speak of "Pferdepotential" but of "Pfedekraft" even though the horse will pull the wagon only if the load is not too heavy.

Suction "tension" (SHULL **36**, p. 214; BECK **1**, p. 425) has the correct dimensions, and is physically more nearly correct than suction "force" (*cf.* also URSPRUNG **45**). If a new term had to be chosen, one would choose tension (Spannung); but since the term suction "force" is already a half century old and has given rise to no misconceptions, it may as well, as I see it, be retained in the future. Even the physicists tolerate a similar liberty in the use of terms, *e.g.*, using "Dampfspannung" instead of "Spannkraft" of steam (CHWOLSON **5**, p. 709); but if Spann"kraft" is permitted in physics, why should the term Saug"kraft" be forbidden in physiology? Furthermore, so long as we retain such terms as cryptogams, tracheae, and leucoplasts one has no right to forbid suction force. It is after all simply a matter of taste whether one, with deference to the historical development, continues to use an old term which has given rise to no misconceptions, or whether one prefers a faultlessly correct expression.

(d) *Counter suggestions for "suction force."*—"Suction" (SIERP in STRASSBURGER's Lehrbuch, 18th ed., p. 76) is a short and clear term but appears to have greater difficulty in becoming established than does suction force or suction tension.

"Water absorbing power" (THODAY **43**, p. 110) does not have the correct dimensions, and is rather lengthy.

"Pouvoir osmotique" (LECLERC DU SABLON **25**, p. 24) reminds one of PFEFFER's "osmotische Triebkraft" (**32**, pp. 76, 84) but it does not indicate whether it signifies suction or pressure.

Concerning the terms "Hydratur" and "Wasserzustand" the reader is referred to section (2) later.

"Turgor deficit" employed by CURTIS (private communication from BECK), it seems to me, can mean only one thing, *i.e.*, a difference between the maximum turgor pressure which is possible and the pressure which actually exists. As is shown in figure 1, the turgor deficit of our *Impatiens* cell is $9.3 - 5.4 = 3.9$ atm., while the suction force of the cell is $S_{z_n} = 4.3$ atm. Turgor deficit and suction force of the cell are not equal numerically, nor are the terms conceptually alike; they cannot therefore be used synonymously.

SHULL (**37**) "sees no good reason for using any other term than osmotic pressure in connection with the turgidity of plant cells." But how, then, shall our osmotic quantity 4.3 atmospheres be designated? The "osmotic pressure" is really 9.7 atm., the "turgor pressure" 5.4 atm.; accordingly the difference, if we are to preserve both these terms, must be designated as "the difference between the osmotic pressure and the turgor-pressure," a term surely too detailed to find any support.[5]

"Traction" was suggested (LIVINGSTON, private communication to BECK, 1927) presumably to replace tension, but I have no further information concerning it.[6]

(e) *Other suggestions.*—Distinctions have been made between static and kinetic, between potential and actual (OPPENHEIMER **30**, **31**; RENNER **35**), between absolute and relative suction force (HUBER **22**; BENEKE-JOST **3**, vol. I, p. 57), and between osmotic and swelling (imbibitional) suction force.

In water intake by the cell osmotic and swelling forces may be associated; the former are usually determinative. Both components in a state of equilibrium must of course be equal to one another: a change in one necessarily causes a corresponding change in the other.

The *Impatiens* cell (fig. 1) with its suction force, $S_{z_n} = 4.3$ atm., can take in water, exude water, or be in equilibrium with the environment. Which case actually obtains depends upon the value and the direction of the suction force gradient (Saugkraftgefälle) of the environment of the cell, as well as upon the resistance to transfusion (Filtrationswiderstand). Just as one in physics speaks of kinetic and static, of actual and potential energy, according to whether or not it results in motion, so one may of course do the same thing in physiology.

[5] SHULL has suggested "net osmotic pressure" for this quantity.

[6] Translator's note: The author refers to a letter from LIVINGSTON to the translator January 3, 1927, in which LIVINGSTON suggested in an informal way a number of terms which were submitted by the translator, of his own accord, to URSPRUNG. The discussion is too lengthy to be given here.

The only question is whether, to avoid misunderstanding, it is necessary to speak of a static suction force when the osmotic energy remains potential, and of a kinetic suction force when the osmotic energy becomes actual and sets the water in motion. An example from mechanics may serve to elucidate this. Suppose that two similar, one-horsepower tractors are attached to the same vehicle; in one case the tractors pull in the same direction and in the other they pull in opposite directions. It is quite unnecessary to refer in the first case to kinetic or actual horsepower and in the second to static or potential horsepower; similarly it is altogether superfluous to make a like distinction in physiology.

Moreover, the expressions *absolute* and *relative* suction force which are employed to signify that the cell with a suction force of 4.3 atm. is in the first case sucking against pure water, and in the second case against an osmotically active environment, are to say the least superfluous. As has already been shown (URSPRUNG and BLUM **49**, p. 2), the suction force is the same in both cases; the *suction force gradient,* however, is different in the environment of the cell. This fact is simply and unequivocally expressed by the use of this old term (suction force).

New and unnecessary terms should, in my opinion, be avoided, as they mean a useless complication of nomenclature which unnecessarily makes understanding difficult for the beginner and layman.

To summarize, then, for the quantity $S_{z_n} = 4.3$ atm. the following terms are recommended: suction force of the cell, suction tension of the cell, or suction of the cell; in addition one may use the expressions suction force gradient, suction tension gradient, or suction gradient.

(2) SUCTION FORCE OF THE CELL CONTENTS, $S_{i_n} = 9.7$ ATM.—What has just been said regarding suction force of the cell applies as well to the discussion of the cell contents. We may therefore give our attention immediately to the counter suggestions.

(a) *Counter suggestions.*—Osmotic pressure is an excellent term in the field of physics, which, however, has not proved adequate in plant physiology. Before 1916 all of the osmotic quantities measured or used were simply referred to as osmotic pressure, even when they differed in numerical value as well as in their concepts. For example, previous to that time osmotic pressure was sometimes understood to mean $S_{z_n} = 4.3$ atm., sometimes it meant $S_{i_n} = 9.7$ atm., sometimes $T_n = 5.4$ atm., sometimes $S_{z_g} = 10.5$ atm. That such a state of affairs must lead to serious confusion is self-evident.

If the physiologist wishes to employ the term "osmotic pressure" he must use it in the sense of the physicist, *i.e.,* the maximum pressure which the cell sap can sustain in an osmometer which is provided with a semi-

permeable membrane. Accordingly it is correct to say that the "osmotic pressure" of the cell sap is 9.7 atm. Let us now examine this term and see whether it serves the physiologist's purpose.

Referring to figure 2, *b,* note that the 0.355 mol cane sugar solution exercises a *suction* upon the water which lies on the opposite side of the membrane and above the mercury, which amounts to 9.7 atm. In figure 2, *a,* the manometer shows a *pressure* of 9.7 atm. The 9.7 atm. may be regarded as a tension as well as a pressure. The cell which we have been considering possesses a turgor pressure of 5.4 atm. as well as an osmotic pressure of 9.7 atm.; since both pressures are the immediate result of the osmotic phenomenon, they may, though distinct, readily be confounded. Let a recent case serve as illustration. (For less recent cases *cf.* URSPRUNG and BLUM **48.**) WENT (**55**) says: "The osmotic pressure of the contents of the cell is received by the stretched cell wall." "Osmotic pressure" is spoken of, which in the case of our illustrative cell is 9.7 atm., while the author has in mind turgor pressure which is really 5.4 atm.

Since the 9.7 atm. (refer to equation $S_{z_n} = S_{i_n} - W_n$) tend *to carry the water into the cell* and the 5.4 atm. tend *to force water from the cell,* it does not seem desirable to apply the same term "pressure" indiscriminately in both cases. Since there is no opposition to the term "turgor pressure" as here used, there remains nothing else but to drop the expression "osmotic pressure" if we are to avoid being misunderstood.

"Osmotic value" was suggested as a substitute for "suction force of the contents of the cell" by HÖFLER (**21**) and WALTER (**53**). The latter desires that the osmotic value should be expressed only in atmospheres, while I express it in molal units.

Under the caption "value" anything may be understood *a priori.* If, however, we are to give unequivocal expression to what we mean, we should agree upon one mode of expression of the quantity (*cf.* also sec. 4). WALTER's suggestion, to express the value only in atmospheres, is not practicable; first because we frequently need the molal expression (*e.g.,* in the equation $O_n = O_g \frac{V_g}{V_n}$, which is necessary for the determination of S_{i_n} of the individual cell), and then because it is not always possible to translate the molal value into atmospheres partly because the concentration data are insufficient. As we already have the expression "suction force (suction tension, suction) of the contents of the cell," the simplest course would be to continue to express the "osmotic value" in molal units.

"Osmotic concentration" has been used for a long time by HARRIS and his associates (**14–19**). Recently PRINGSHEIM (**33**) also has suggested the term as a substitute for "osmotic pressure." DIXON and ATKINS (**7**) as also KORSTIAN (**24**) speak of "sap concentration." These designations

are not to be recommended as substitutes for the quantity S_i, which is here in question, for it must be measured in atmospheres (as follows consequentially from the equation $S_z = S_i - W$). Logically a concentration should not be expressed in atmospheres.

"Density of cell sap" is used by KORSTIAN (**24**) as synonymous with "osmotic pressure." This expression also is unsuitable since density cannot be measured in atmospheres.

"Water relations," "water conditions," and "Hydratur" are expressions that WALTER (**52–54**) suggests as substitutes for "suction force (suction tension, suction) of the contents of the cell" and he expresses the quantity in atmospheres. He uses three methods for the measurement of "Hydratur" which yield results that are numerically different and are altogether different in concept: (1) Cryoscopy of the expressed sap which gives an average value of $S_{i_n} = 9.7$ atm.; (2) Determination of the vapor pressure of intact cells which gives values of $S_{z_n} = 4.3$ atm. and not values for S_{i_n}; (3) The method of incipient plasmolysis by which $S_{i_g} = S_{z_g} = 10.5$ atm. is determined. Not only do these methods yield quantities that are altogether different in themselves but they are rechristened and defined in a manner that introduces confusion. While WALTER thinks he is measuring the "water condition" or "Hydratur," usually by determining the quantity $S_{i_n} = 9.7$ atm., RENNER (**35**) explains that the "water condition" or "Hydratur" can agree in value only with the suction force of the cell $S_{z_n} = 4.3$ atm. This confusion follows from the fact that both authors are measuring different quantities and apply common names indiscriminately. WALTER studies the situation from the point of view of the suction force of the protoplasm, while RENNER regards it from the point of view of the suction force of the cell. These recent examples show how futile it is to form a new, indefinite and unnecessary terminology; it makes things very difficult for those who are not very familiar with the subject, while nothing of value is gained. I retained, whenever it was at all possible, the already-existing terms, precisely for the purpose of not overburdening the nomenclature.

(3) THE SUCTION FORCE AT INCIPIENT PLASMOLYSIS, $S_{i_g} = S_{z_g} = 10.5$ ATM.—Here again that which was already mentioned about "suction force" holds, *i.e.*, that "suction tension" or "suction" should be considered synonymous with it. The modifications "of the cell" and "of the contents of the cell" become superfluous, since $S_{i_g} = S_{z_g} = 10.5$ atm. At this time it is probably self-evident that only the suction force at incipient plasmolysis can be measured and not all other kinds of quantities. It follows that previous misunderstandings in so far as they arose from confusion of terms should henceforth be eliminated. The determination of $S_{i_g} = 10.5$ atm. by the plasmolytic method has rendered physiology great service (one has only to

recall the work of DE VRIES), and will continue to do so in the future (*e.g.*, in the study of osmotic regulation) if one only uses a proper plasmolytic agent, and if care is taken to interpret the results correctly.

(4) THE OSMOTIC VALUE, $O_n = 0.355$ MOL CANE SUGAR.—

(5) THE OSMOTIC VALUE AT INCIPIENT PLASMOLYSIS OR INCIPIENT PLASMOLYSIS VALUE, $O_g = 0.38$ MOL CANE SUGAR.—

The term "osmotic value" (when the attribute is lacking, "in the normal phase" is understood) signifies the molal expression of the concentration of the plasmolyte which is isotonic with the cell sap, when the cell has the normal volume (URSPRUNG and BLUM **48**; BECK **2**). The osmotic value at incipient plasmolysis, $O_n = 0.38$ mol cane sugar, must first be determined before the quantity $O_n = 0.35$ can be deduced from it. The deduction is made by means of the equation, $O_n = O_n \frac{V}{V_n} = 0.355$ mol cane sugar, in which V_g is the volume of the cell at incipient plasmolysis, and V_n the volume in the normal phase.

(a) *Other suggestions*.—HÖFLER (**21**) made the suggestion which was seconded by HUBER (**23**) that the osmotic value should be expressed in atmospheres as well as in molal units. According to this suggestion we should write: $O_n = 0.355$ mol cane sugar $= 9.7$ atm.

As previously mentioned, the term value may, *a priori*, be variously employed, but in the interest of a simple nomenclature, which admits of but one interpretation, we should agree upon some simple but adequate term. Furthermore, a 0.355 molal cane sugar solution can only be equivalent to but not identical with a pressure of 9.7 atm. Different quantities measured in different systems of units may not be given a common name just because they have equivalent values. Now if the quantity measured in atmospheres be designated as the suction force (suction tension, suction) of the contents of the cell, there is nothing to prevent us from calling the quantity with its equivalent values, expressed in molal concentration units, the osmotic value.

Osmotic concentration.—PRINGSHEIM (**33**) suggests that the term "osmotic value" be rejected as not sufficiently definite, while WALTER (**53**, p. 83) wishes to have the quantity introduced even into the suction-force equation. Let it be recalled that just as in the case of the term suction force, we are dealing with a *time-honored* term that for long has been employed in plant physiology. It harmonizes very well, too, with the ordinary modes of speech; for just as we speak of the monetary value of various things we may discuss the osmotic value of different cell saps. Accordingly it is certainly permissible to speak of the "osmotic value" as being 1 mol cane sugar solution. The substitute which PRINGSHEIM (**33**) proposes, "osmotic concentration" of 1 mol cane sugar solution, is decidedly not an improvement. In the first place the new term may give the impression that

the cell sap contains a 1 mol cane sugar solution; then again the concentration is measured in mols but not necessarily with cane sugar; finally the concentration is not the only factor to be considered in the osmotic phenomena for the chemical constitution of the sap is a factor too.

(6) THE WALL PRESSURE $W_n = 5.4$ atm.—

(7) THE TURGOR PRESSURE $T_n = 5.4$ atm.—

From the nature of things it follows that the wall pressure is the pressure that is exerted by the wall upon the contents of the cell. Turgor pressure is generally admitted to be the pressure exerted by the contents of the cell upon the wall. In a condition of equilibrium $T = W$, when no foreign forces enter the consideration. If foreign forces are to be considered, $T = W \pm A$. I am not aware of any terms that may have been suggested as substitutes for these.

(b) *The suction-force (suction tension) equation.*—My equation reads: The suction force of the cell = the suction force of the contents of the cell − the wall pressure; $S_z = S_i - W$; of course suction force may be replaced by suction tension or by suction. If foreign forces enter into consideration the equation becomes: $S_z = S_i - W \pm A$. T is given by $W \pm A$.

Suggested substitutes.—RENNER (**34**): Saugkraft der Zelle = osmotische Druck − Turgordruck.

$$S = P - T.$$

This equation was published before mine; both were established independently.

THODAY (**43**): Water absorbing power = osmotic pressure − turgor pressure.

$$p = P - T.$$

HÖFLER (**21**): Saugkraft der Zelle = osmotischer Wert (in Atm.) − Turgordruck.

$$S = O - T.$$

LECLERC DU SABLON (**25**) pouvoir osmotique du liquide exterieur = pouvoir osmotique du suc cellulaire − turgescence.

STILES (**40, 41**): suction pressure = osmotic pressure − wall pressure.

$$S = P - T.$$

WALTER (**53**): Saugkraft = osmotischer Wert (in Atm.) − Wanddruck.

SIERP (**39**): Saugung der Zelle = Saugung des Zellinhaltes − Wanddruck.

Several of the suggested substitutes place turgor pressure in the place of wall pressure in my equation; I do not recommend this form of writing the equation as it does not indicate the actual condition. The quantity which acts counter to the intake of the water is not the turgor pressure but the wall pressure (ev. $W \pm A$).

Other variations often consist in replacing S_i by the osmotic pressure. As was shown previously (2) this is not to be recommended; first because it leads to confusion with the turgor pressure, and then again because it is to no good purpose to use the common term "pressure" for a quantity which tends to press water from the cell and another which tends to draw water into the cell.

The why and the wherefore of expressing the osmotic value in molal units rather than in atmospheres has been discussed (*cf.* sections 2, 4, 5).

If suction tension or suction is to be preferred to suction force it is a matter of taste so far as I am concerned.

(c) *Suggestions on the reduction of the number of terms.*—As was mentioned above, SHULL (**36–38**) prefers to use no term other than "osmotic pressure" in connection with the turgidity of plant cells. Similarly LUBIMENKO (**27**) speaks only of "pression osmotique" and "pression de turgescence."

HÖBER (**20**) speaks of "osmotischen Druck" and "Turgor." OLTMANNS (**29**) even tries to get along with the term "turgor" alone.

In itself, of course, the notion of reducing the number of terms is very welcome; but for all that, the basic purpose, *i.e.*, the possibility of clear expression of ideas and the elimination of misunderstandings, must not be sacrificed. That two terms will not suffice to express unequivocally seven quantities will probably not be questioned in view of the illustrations which were drawn from various authors, and further demonstration will hardly be necessary. Referring again to SHULL's reviews, he wrote (**36**) in reference to BLUM's measurements of suction force in alpine plants: "These suction force studies show in a different way by plasmolytic means, just what HARRIS's studies of freezing point depressions have shown, a general correspondence of plant cells to the conditions of the habitat." If he intends to convey the notion that the same quantity was measured in two different ways, he is in error.[7] HARRIS—referring once again to the illustrative cell of *Impatiens*—measured $S_{i_n} = 9.7$ atm.; BLUM measured $S_{z_n} = 4.3$ atm. The fact is that the two quantities usually do vary in the same sense. Thus while passing from the phase of incipient plasmolysis to the saturation phase (*cf.* fig. 1), both experience a decrement, but it is slight in S_i, *i.e.*, from 10.5 to 9.3 atm., and considerable in S_z, *i.e.*, from 10.5 to 0.0 atm. If the quantity S_{i_n} is determined in the study of the water economy, faulty individual values are obtained, because the real indicator of the conditions is S_{z_n}.

Whoever attempts to carry on with only one or two terms is bound to fall into the same errors that were committed in the past, *i.e.*, labeling indiscriminately $S_{z_n} = 4.3$ atm., $T_n = 5.4$ atm., $S_z = 10.5$ atm., whichever quantity is desired, as "osmotic pressure."

[7] He had no such intention.

Summary

The earlier studies of osmosis in plants led to confusion because a common name was applied to different quantities and because of attempts to measure these quantities by a common method. The creation of methods which permit the determination of the different quantities numerically as well as in concept, constitutes the essential difference between the more recent studies and the older ones. In order to avoid misunderstandings a new terminology became necessary. It embraces the expressions: Suction force (suction tension, suction) of the cell, S_{z_n}; suction force (suction tension, suction) gradient; suction force (suction tension, suction) of the contents of the cell, S_{i_n}; suction force (suction tension, suction) at incipient plasmolysis $S_{z_g} = S_{i_g}$; osmotic value, O_n; osmotic value at incipient plasmolysis = incipient plasmolysis value, O_g; wall pressure, W_n; turgor pressure, T_n; and the turgor distention produced by the turgor pressure. The terms are, as they should be, unequivocal, simple, and easy to understand. In so far as it was possible, they were linked to the old terminology. The terms suction and pressure were chosen in accord with the indications on the manometer. Wherever the manometer permits both designations, the form that seems best adapted to avoid misunderstandings has been chosen. The prime purpose of terminology is simplicity and the elimination of error. When these ends can be obtained in different ways a certain flexibility in the mode of expression should be tolerated. With me it is a matter of taste whether suction tension or suction be considered synonymous with suction force. Even though it is desirable to use but a single term for a given quantity, it is not absolutely necessary, so long as the essential purpose of terminology is not defeated. For decades the significance of terminology was underestimated, but we need not for all that go to the other extreme.

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DETERMINATION OF AMINO NITROGEN IN PLANT EXTRACTS

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Introduction

In nitrogen partition studies it is customary to estimate the amount of nitrogen contained in the alpha-amino groups of amino acids by means of the Van Slyke gasometric or the Sörensen formol titration method. As is generally known, these methods were developed for the analysis of pure amino acids or products of protein hydrolysis as applied particularly to animal tissues. Since plant extracts usually contain water soluble pigments and buffering substances which interfere with the formol titration, the Van Slyke method is more frequently employed.

While engaged in some nitrogen partition studies with alcoholic extracts of apple leaves (9), the writer observed that the yield of amino nitrogen by the standard Van Slyke procedure was in excess of the total soluble, non-protein nitrogen. This finding has repeatedly been confirmed and the same error noted in varying degrees in several other plant tissues extracted with water or 80 per cent. alcohol. The present investigation was undertaken to study the factors responsible for these anomalous results, and to develop, if possible, corrective measures.

Materials and methods

Plant tissues used in this study included: apple leaves; rhubarb petioles and leaves; clover roots and tops; young cabbage; tomato, soy bean, and sunflower plants; begonia petioles and leaves; and potato tubers.

ALCOHOLIC EXTRACTION OF SOLUBLE NITROGENOUS SUBSTANCES.—Representative 100-gm. samples of tissue were preserved in sufficient hot 95 per cent. alcohol to give a final concentration of 80 per cent. The alcohol was filtered off and the residue extracted eight times with 80 per cent. alcohol by the decantation method (9).

WATER EXTRACTION OF SOLUBLE NITROGENOUS SUBSTANCES.—Similar samples of fresh tissue were passed through a Nixtamal mill and weighed portions of the pulp were ground in a mortar with nitrogen-free sand and water at a temperature of 25° C. The soluble material was expressed by hand through finely woven cloth. The residue was again ground in the mortar and the process repeated until complete extraction of the soluble, non-protein nitrogenous substances was obtained.

Analytical procedure

TOTAL NON-PROTEIN NITROGEN.—Total nitrogen of the protein-free extracts was determined by the Kjeldahl method. When it was possible to

demonstrate the presence of nitrates by the diphenylamine test, the modified method of PUCHER *et al.* (**6**) was used.

ALPHA-AMINO NITROGEN.—All amino determinations were made with the Van Slyke micro-apparatus, using 2- or 4-ml. aliquots. Blank determinations and the proper corrections for temperature and pressure were made regularly. Deaminization was allowed to proceed for a period of five minutes and the usual precautions to insure accurate comparable results were consistently observed. Values reported for amino nitrogen are averages of duplicate determinations which agreed closely.

Difficulty was encountered in obtaining a satisfactory anti-frothing reagent for use during deaminization. Several samples of caprylic alcohol proved unsatisfactory owing to the formation of a large blank. A number of other materials were tested in blank determinations and with plant extracts. Of these toluene proved the best. When used in 0.25-ml. portions the troublesome frothing was largely suppressed.

Experimental results

SUBSTANCES IN PLANT EXTRACTS CAUSING ERRORS IN THE VAN SLYKE METHOD

AMMONIA.—It is well known that ammonia reacts with nitrous acid in the amino determination, liberating free nitrogen. VAN SLYKE reported (**13**) that 36.3 per cent. of the ammonia contained in an M/5 solution of ammonium sulphate was liberated in five minutes by nitrous acid in the macro-apparatus. It seemed desirable to repeat this experiment under the conditions encountered in this study. Solutions containing 1.0, 0.5, 0.25, and 0.125 mg. of ammonia per ml. were prepared from ammonium sulphate and 4-ml. aliquots analyzed in the micro-apparatus. It was established that the yield of nitrogen was a linear function of the concentration of ammonia and approximately constant under a given set of conditions. Thus in one set of experiments, conducted at a temperature of 30° C. and barometric pressure of 765 mm., an average of 35.2 per cent. of the ammonia was obtained as amino nitrogen following a 5-minute deaminization period. This percentage recovery was found to vary from time to time, being influenced apparently by temperature, time, rate of deaminization, etc. Accordingly it is doubtful whether attempts to apply a corrective factor for the ammonia present in solutions analyzed will prove uniformly satisfactory. Hence the removal of free ammonia, if present in appreciable quantities, is necessary for accurate amino determination.

It is generally considered that only small amounts of ammonia are found in plant tissues. In certain acid plants, however, as the begonia and rhubarb, ammonia may constitute a large proportion of the total soluble, non-protein nitrogen. Obviously any amino determinations made on these extracts would be greatly in error if the ammonia were not previously re-

moved. It is of interest to note that CULPEPPER and CALDWELL (3), who have recently studied the nitrogen metabolism of rhubarb in some detail, encountered difficulty in interpreting the results of their amino determinations. These investigators attributed the irregularities and fluctuations of their data to the effect of preservation and storage of the tissue in alcohol. While this is undoubtedly a factor, the ammonia content of the tissues, which apparently was not considered, would vitiate the results of the amino determinations if the rhubarb used in the present study was at all comparable.

Frequently plants grown under disturbed nutritional conditions are used for analytical studies. Under such conditions ammonia accumulation often occurs as a result of a breakdown in the nitrogen metabolism. Ammonia may also increase in stored alcoholic extracts of plant tissues, according to WEBSTER (15). For these reasons some investigators, as THOMAS (11), CHIBNALL (2), NIGHTINGALE (4), and others, have employed methods whereby the ammonia is removed prior to the amino determination. This may be accomplished by a preliminary distillation to remove the free ammonia. In some instances the amide linkages are hydrolyzed and the total ammonia is then removed prior to making the amino determinations. This procedure measures all of the free alpha-amino groups, but some hydrolysis of peptide linkages may have occurred during the amide hydrolysis. Frequently the filtrate from the phosphotungstic acid precipitate of basic nitrogen is analyzed for amino nitrogen. This filtrate contains only the mono-amino acids and simple peptides.

DERIVATIVES OF PHENOLS.—Investigation disclosed that the di- and trihydric phenols and their acid derivatives (as the tannins) were readily oxidized in the Van Slyke determination with the formation of gases difficultly soluble in the alkaline permanganate. The amount of this gas decreased somewhat upon long continued contact with the permanganate. The amount of gas formed seemed to be correlated with the number and position of the hydroxyl groups present in the compound. Thus the trihydric phenol, phloroglucinol, yields considerably more gas than the dihydric resorcinol. When the hydroxyl groups are present in the ortho position, as in catechol or pyrogallol, much less of the insoluble gas is produced. Intermediate values were obtained for hydroquinone which has the para grouping of the hydroxyls. Ordinary phenol has little effect. It would therefore be expected that the compounds which occur in plants as esters and glucosides would react with nitrous acid according to the type of linkage, number of free hydroxyl groups, orientation of these groups, and probably other factors which are still obscure.

The tannins, which may be regarded as phenol acids, or glucosides of these acids, are widely distributed in plant tissues. Pure tannic acid

(Merck's) reacts readily with nitrous acid, forming a gas which would be measured as nitrogen in the amino determination. Extracts of oak galls known to contain large quantities of tannin were also found to react in the same manner. Many other so-called secondary plant substances are known to contain phenolic groups. Such a compound, for instance, is the glucoside phlorizin in which phloroglucinol occurs as an ester. This material is known to be present in apple tissue in large quantities. Pure phlorizin was found to react in the amino determination with the formation of small quantities of insoluble gas.

Miscellaneous substances.—A further source of error lies in the compounds extracted with 80 per cent. alcohol but insoluble in water. When these compounds, consisting of pigments, lipides, and other material of indefinite composition, are suspended in water and introduced into the Van Slyke apparatus, a variable amount of gas insoluble in the alkaline permanganate is obtained. It is, however, a simple matter to remove these materials by the addition of a small amount of toluene or chloroform to the concentrated water suspension remaining after the removal of the alcohol. The writer prefers chloroform since it sinks to the bottom of the flask, carrying the lipides with it and permitting the clear supernatant extract to be withdrawn. The possibility must be kept in mind that other chemical groups may react with nitrous acid, reducing it to nitrogen gas or liberating other insoluble gases. Tangible evidence for this suggestion is afforded by the well known fact that ethyl alcohol and acetone form large quantities of gas when subjected to the conditions of the amino determination.

Procedures to reduce errors in the Van Slyke method when applied to plant extracts

In order to discover the most satisfactory method to reduce errors in the Van Slyke method when applied to plant extracts, several plant tissues were extracted with water and with 80 per cent. alcohol, and the extracts were subjected to different treatments prior to the determination of amino nitrogen in the extracts. The soluble proteins were removed from the water extracts with colloidal ferric hydroxide as described by Thomas (10). The pigments, lipides, etc., were removed from the alcoholic extracts by adding chloroform to the alcohol-free filtrates. The emulsion was flocculated with magnesium sulphate as previously described (9).[1] Suitable aliquots of these partially cleared water and alcoholic extracts were then subjected to further treatments.

[1] This method was originally suggested by Dr. T. G. Phillips of the University of New Hampshire.

PRECIPITATION WITH NEUTRAL LEAD ACETATE SOLUTION.—One lot of aliquots was further cleared with saturated neutral lead acetate. The excess lead was removed with anhydrous sodium oxalate.

ADSORPTION WITH DECOLORIZING CARBON.—A second lot of aliquots was stirred at intervals with decolorizing carbon for one hour, filtered, washed, and the filtrates concentrated. The degree of adsorption is admittedly indefinite and probably depends upon the concentration of adsorbent and extract and the time. As far as possible these factors were kept constant.

ADSORPTION WITH CALCIUM OXIDE.—To a third lot of aliquots sufficient calcium oxide was added to make the extract slightly alkaline. The mixture was stirred at intervals for one hour, filtered, washed, and the filtrate concentrated after making faintly acidic with acetic acid.

DISTILLATION WITH SOLID CALCIUM OXIDE.—Distillation of a fourth lot of aliquots with solid calcium oxide under reduced pressure for one hour at 40°–45° C. was carried out in the usual VAN SLYKE ammonia apparatus (**12**). A slight excess of calcium oxide was used as determined by preliminary titration of a small portion of the extract. The extract was filtered from the calcium, washed, acidified with acetic acid, and concentrated. The ammonia was collected in 0.02 N acid and titrated to a methylene blue-methyl red indicator with 0.02 N base. This method was developed by PLIMMER and ROSEDALE (**5**) to permit controlled alkalinity in the determination of ammonia by the Van Slyke method. The same method was later applied to extracts of apple wood and leaves by THOMAS (**11**), who noted that this method, besides removing the ammonia, adsorbed the materials which cause frothing during deaminization. The results of the present study have fully confirmed the findings of these investigators, and in addition have shown that this distillation results in a decrease in the amount of gas measured as amino nitrogen. The results of these preliminary analyses are shown in tables I and II.

The data in tables I and II indicate that all treatments resulted in a decrease in amino nitrogen as compared with the direct determination on the extracts which were only partially clarified in removing the alcohol, lipides, protein, etc. This decrease was fairly uniform for a given tissue when treated with the precipitating and adsorbing agents, but in no instance was it so great as that obtained by distillation. The latter method was accordingly selected for further study. The pronounced difference between the amounts of nitrogen extracted by water and by alcohol is typical of many analyses and will be discussed in a later section of this paper.

The possibility must be kept in mind that the distillation procedure as previously described may result in a loss of amino acids through occlusion or other means, thereby accounting for a portion of the reduction in yield. Since there are no methods for determining the absolute amount of any

TABLE I

AMINO NITROGEN IN WATER EXTRACTS OF PLANT TISSUES AFTER DIFFERENT TREATMENTS OF THE EXTRACTS

TREATMENT OF EXTRACTS	WATER EXTRACTS OF 100 GM. OF FRESH TISSUE		
	APPLE LEAVES	TOMATO PLANTS	POTATO TUBERS
	AMINO N	AMINO N	AMINO N
	mg.	*mg.*	*mg.*
Colloidal ferric hydroxide to remove soluble proteins	80.3	38.5	148.0
Further treatments of protein free extracts:			
Neutral lead acetate	34.1	33.1	143.3
Carbon	30.7	33.3	140.7
Calcium oxide	33.5	33.4	137.6
Distillation with calcium oxide	22.4	30.5	123.8

TABLE II

AMINO NITROGEN IN 80 PER CENT. ALCOHOLIC EXTRACTS OF PLANT TISSUES AFTER DIFFERENT TREATMENTS OF THE ALCOHOL-FREED EXTRACTS

TREATMENT OF EXTRACTS	ALCOHOLIC EXTRACTS OF 100 GM. OF FRESH TISSUE		
	APPLE LEAVES	TOMATO PLANTS	POTATO TUBERS
	AMINO N	AMINO N	AMINO N
	mg.	*mg.*	*mg.*
Chloroform to remove pigments, lipides	44.0	27.7	83.5
Further treatments of pigment and lipide free extracts:			
Neutral lead acetate	19.5	23.2	78.2
Carbon	18.5	21.8	76.0
Calcium oxide	20.7	21.6	78.2
Distillation with calcium oxide	14.8	20.4	70.2

plant constituent, this question cannot be definitely answered. However, numerous tests with the pure amino acids, leucine and arginine, and the half-amide of aspartic acid, asparagine, resulted in quantitative recovery after the distillation. Pure amino acids could also be quantitatively re-

covered when added to plant extracts which had previously been distilled with calcium oxide and analyzed for amino content. Furthermore, when the amino acid solutions were added to the partially clarified but undistilled plant extracts, the theoretical value could be obtained by analysis after distillation. Their value was determined by distilling other aliquots of the same extract without addition of amino acids and noting the decrease in amino value.

Quadruplicate determinations on individual extracts were found to agree closely. Certain extracts were redistilled and it was established that no further decrease occurred after the first treatment. On the basis of these studies it is concluded that treatment with calcium oxide has no effect on the amino acids contained in plant extracts. The decrease in yield must then be due to other factors.

The calcium oxide residue remaining after distillation was subjected to study. It was found possible to remove the adsorbed material with acetic acid, and after neutralization it could be analyzed directly. Small amounts of gas measured as nitrogen in the Van Slyke determination were obtained which varied with different extracts. The amounts, however, could not account for the magnitude of the reduction in amino nitrogen brought about by distillation. The distillation must have exercised some denaturing effect on the interfering substances. Aside from precipitating or occluding certain materials on the surface of the calcium oxide, an oxidizing effect should be obtained as well, since a stream of air is drawn through the suspension. Under such conditions it was observed that tannic acid as well as the tannin from oak galls could be quantitatively precipitated or denatured so that no gas insoluble in alkaline permanganate was liberated in the amino determination. Distillation with calcium oxide was therefore considered to be the best method since its use removes the free ammonia, adsorbs the materials which cause frothing during deaminization, and precipitates or denatures the tannins.

After this investigation had been completed the work of RAHN (7) came to the writer's attention. This worker noted that when plant extracts were treated with tannic acid to precipitate the soluble protein, difficulty was encountered in making the amino determination by the Van Slyke method. The difficulty was attributed to a reduction of nitrogen trioxide, N_2O_3, said to arise in the determination, to elemental nitrogen. It is generally considered that nitric oxide, NO, rather than N_2O_3, arises in the determination. The latter compound is formed only at low temperatures from NO and NO_2 and at room temperature would not exist. RAHN reported that the tannic acid could be satisfactorily removed by allowing the extract to stand one day with a concentrated solution of potassium bichromate. Tannic acid is seldom used as a protein precipitant in this country. The widespread

occurrence of tannins in plant tissue, however, makes it imperative that their effect be removed before estimation of amino nitrogen. This is particularly important when tissues containing but a small amount of amino nitrogen are analyzed.

Limits of error in amino nitrogen determination in different tissues

In order to gain some information concerning the limits of error in amino nitrogen that may normally occur in tissues frequently analyzed, a number of such tissues were extracted with water and 80 per cent. alcohol and the amino content estimated before and after distillation with calcium oxide. The total soluble, non-protein nitrogen was also determined. The results appear in table III.

Without exception distillation with calcium oxide reduced the amount of "amino" nitrogen ranging from 6.1 to 84.9 per cent. of the values deter-

TABLE III

Total soluble, non-protein nitrogen, and amino nitrogen in various plant extracts before and after distillation with calcium oxide. Results expressed as mg. per 100 gm. of fresh tissue

Tissue	Method of extraction	Soluble non-protein	Amino nitrogen in plant extracts			
			Before distillation	After distillation	Decrease	Percentage decrease
		mg.	mg.	mg.	mg.	%
Cabbage plants	80% alcohol	23.8	14.3	10.8	3.5	24.5
	Water	51.5	23.6	19.3	4.3	18.2
Clover tops	80% alcohol	75.6	32.8	29.2	3.6	11.0
	Water	133.3	47.9	44.6	3.3	6.9
Clover roots	80% alcohol	117.2	48.7	41.7	7.0	14.4
	Water	170.2	60.7	56.2	4.5	7.4
Soy bean plants	80% alcohol	105.7	43.0	39.0	4.0	9.3
	Water	163.0	55.4	52.0	3.4	6.1
Sunflower plants	80% alcohol	26.9	6.1	4.6	1.5	24.6
	Water	34.7	10.9	9.5	1.4	12.8
Begonia petioles	80% alcohol	24.3	9.3	1.4	7.9	84.9
	Water	42.9	11.3	3.9	7.4	65.5
Begonia leaves	80% alcohol	15.4	8.6	3.9	4.7	54.7
	Water	32.2	10.6	5.5	5.1	48.1
Rhubarb petioles	80% alcohol	65.0	17.4	3.2	14.2	81.6
	Water	77.8	19.1	6.7	12.4	64.9
Rhubarb leaves	80% alcohol	59.0	24.6	10.6	14.0	56.9
	Water	99.3	30.3	18.1	12.2	40.3

mined before distillation of the extracts with calcium oxide. Distinct tissue differences are evident which must depend upon the amount of non-amino substances free to react in the determination. In most cases good agreement is evident between the decrease in amino value of both water and alcoholic extracts of the same tissue, in spite of the fact that greater amounts of amino nitrogen were invariably removed by the water. Since the size of the aliquot distilled represented a much greater proportion of the total ex-

TABLE IV

AMMONIA AND AMINO NITROGEN IN EXTRACTS OF NORMAL AND PHOSPHORUS DEFICIENT TOMATO PLANTS. RESULTS EXPRESSED AS MG. PER 100 GM. OF TISSUE, FRESH WEIGHT BASIS

Tissues	Growth conditions	Ammonia in extracts	Amino nitrogen in extracts			
			Before distillation with CaO	After distillation with CaO	Decrease	Percentage decrease
		mg.	*mg.*	*mg.*	*mg.*	%
Upper leaves	Normal	11.7	20.3	14.1	6.2	30.5
	Phosphorus deficiency	22.6	25.9	16.1	9.8	37.8
Lower leaves	Normal	13.5	20.8	14.2	6.6	31.7
	Phosphorus deficiency	19.3	27.2	19.2	8.0	29.4
Upper stems	Normal	19.4	25.4	18.0	7.4	29.1
	Phosphorus deficiency	29.3	31.4	21.2	10.2	32.5
Lower stems	Normal	29.9	40.9	30.1	10.8	26.4
	Phosphorus deficiency	83.3	65.2	37.2	28.0	42.9
Roots	Normal	13.5	20.7	13.7	7.0	33.8
	Phosphorus deficiency	26.4	26.3	17.1	9.2	35.0

tract in the case of the alcoholic extracts than with the water extracts, the possibility of the decrease being due to occlusion or destruction of the amino acids through errors in the determination seems precluded. The percentage decrease of amino nitrogen was always greater in the case of the alcoholic extracts, owing to the smaller original amino content.

The analyses reported in tables I, II, and III were carried out with freshly extracted tissues. Accordingly it seemed desirable to investigate the behavior of plant extracts which had been subjected to typical laboratory con-

ditions. Tomato plants were grown in a complete nutrient solution and in a solution deficient in phosphorus, during the spring of 1932, the continuous renewal type of water culture being used. The plants were sampled for analysis in June, using the alcohol preservation method which has been described. Each series of plants was divided into upper and lower stems, upper and lower leaves, and roots. The primary branches were included with the stems, secondary branches and petioles with the leaves. The samples were extracted eight times by the decantation method during October, 1932. The extracts were made to definite volumes, sealed, and allowed to stand six months. At the end of this period suitable aliquots were withdrawn and analyzed for amino nitrogen before and after distillation with calcium oxide. The amount of ammonia present in the extracts was determined by the SESSIONS and SHIVE aeration method (8). The results are shown in table IV.

A considerable difference exists between normal and phosphorus deficient plants in ammonia content. Indeed the ammonia content of the deficient plants is greater than their amino nitrogen content, which in turn is greater than the amino content of the normal plants. Such a large amount of ammonia would be expected to give erroneous amino values and the data indicate that this is the case, the amount of error ranging from 26.4 to 42.9 per cent. of the direct determination. The amino values after distillation are not greatly different in the normal and in the phosphorus deficient plants, whereas the direct determination exhibited much wider differences, particularly in the lower stems. It is obvious that determining the amino nitrogen in the presence of appreciable amounts of ammonia results in an overlapping of the nitrogen fractions. As a result the residual or "other" nitrogen is too low. At the present time investigators assign considerable importance to this fraction in the interpretation of metabolism studies. In the stems and roots the concentration of ammonia is sufficient to account for the decrease in "amino" nitrogen after distillation. In the leaves, however, other substances must have been present which added to the ammonia error.

Effect of alcoholic storage on amino nitrogen

Recently WEBSTER (14, 15) has reported that stored alcoholic extracts of plant tissue usually increase in ammonia and decrease in amino nitrogen. In WEBSTER'S work the free ammonia was apparently not removed from the extract before making the amino determination. Since the ammonia content of the extracts increased, the direct determination of amino nitrogen should show an increase as well unless the ammonia arises through deaminization of the alpha-amino groups. If the ammonia does increase in this manner, then extracts should show even greater decreases after the removal of this ammonia than when the determinations are made without such treat-

ment. Furthermore, the question naturally arises whether or not the observed decrease in amino nitrogen may be due to transformations of the non-amino reacting substances other than ammonia.

Preliminary experiments designed to throw some light on these questions have been carried out with alcoholic extracts of tomato leaves and stems stored in light and darkness. The results of periodic analyses clearly confirm the conclusions of WEBSTER. In one instance with extracts of tomato stems the amino content decreased 35.8 per cent. in the dark and 47.3 per cent. in the light during a storage period of five months. The ammonia content, estimated before making the amino determinations, steadily increased. The rate of increase in ammonia and decrease of amino nitrogen was most rapid during the first four weeks of storage. The loss of amino nitrogen was far greater than could be accounted for by the increase in ammonia. This decrease must represent an actual change in the amino groups so that they are not free to react with nitrous acid. The mechanism of the change is not clear. It is probable that considerable variation might exist in other tissue extracts. Also the rates might be somewhat different during storage in alcohol before complete extraction. However, the common practice of preserving large numbers of samples which cannot be analyzed for long periods of time must be questioned.

Comparison of water and 80 per cent. alcohol as extractives of soluble nitrogen

The data contained in tables I, II, and III permit a direct comparison to be made between water and 80 per cent. alcohol as extractives of soluble nitrogen and amino nitrogen. Without exception extraction with water removed larger amounts of nitrogen than did alcohol. In fact, the magnitude of the differences raises some question as to the value of 80 per cent. alcohol as an extractive of the nitrogenous substances. In some instances 50 per cent. alcohol has proved to be about as effective as water for extraction of the soluble nitrogen. In nitrogen partition experiments with potato tubers it was established that 97 per cent. of the non-protein nitrogen removed with water could be extracted from similar samples with 50 per cent. alcohol by volume. In such cases the tissue is best preserved in 80 per cent. alcohol, the subsequent extractions being made with 50 per cent. alcohol. It would seem that this method (1) is deserving of further study and use when large numbers of samples must be handled in a limited period of time.

The writer has frequently found that the excess of total soluble, non-protein nitrogen removed with water over that removed with 80 per cent. alcohol is reflected in higher concentrations of all the soluble fractions, with the exception of ammonia or other volatile bases measured as ammonia. This difference is usually most pronounced in the basic fraction. The in-

crease is not due to incomplete removal of the soluble protein from the water extracts, as preliminary experiments with other protein precipitants, acetic acid, trichloracetic, etc., gave entirely similar results. The possibility of proteolytic enzyme activity was restricted to a minimum since in this work duplicate samples of but a single tissue were taken for extraction at a time, thus permitting prompt treatment of the extracts. It is realized that not all tissues in their fresh state lend themselves to extraction with water. However, it seems desirable whenever possible in nitrogen partition studies to employ fresh tissue and water extraction.

Discussion

In this paper evidence has been presented indicating that the conventional methods for the determination of amino nitrogen in plant extracts may give erroneous results. It has been shown that the preliminary distillation with calcium oxide as outlined made possible the determination of amino nitrogen values which more closely approached the true values. The importance of this treatment depends upon the tissue involved. In a comparatively wide range of plant tissues there was a decrease in amino nitrogen when distilled with calcium oxide, but these data must be regarded as relative rather than absolute. While the composition of any species is doubtless fairly well defined, wide fluctuations in the proportions of the various fractions may occur through differences in physiological age, mineral and organic nutrition, etc. That the amount of amino nitrogen as well as of the non-amino substances concerned will be influenced is certain. Aside from the errors involved in the actual amino determination, it is evident that the preservation and extraction of the tissue may greatly influence the results. The necessity of a careful preliminary study of the properties of a tissue before undertaking extensive analytical work must be emphasized.

Summary and conclusions

1. Treatment of plant extracts with neutral lead acetate, decolorizing carbon, and solid calcium oxide invariably resulted in a decrease of gas measured as amino nitrogen by the Van Slyke method. Evidence is presented supporting the validity of the method of low-temperature distillation with solid calcium oxide under reduced pressure, which resulted in maximum decrease in all cases.

2. Acid derivatives of the phenols, as the tannins, were found to react with nitrous acid in the amino determination producing gases measured as nitrogen. This error is eliminated by distillation with calcium oxide. Limits of error caused by the presence of ammonia are discussed.

3. Reductions in gas measured as amino nitrogen ranging from 6.1 to 84.9 per cent. were found in twelve plant tissues extracted with water and

80 per cent. alcohol after distillation with calcium oxide. Five plant fractions of normal and phosphorus-deficient tomato plants showed similar decreases of 26.4 to 42.9 per cent. after ten months' storage in alcohol.

4. Alcoholic storage of tomato plant extracts was found to result in marked increases in ammonia nitrogen and decreases in alpha-amino nitrogen.

5. With nine plant tissues 80 per cent. alcohol extracted an average of only 63.7 per cent. as much soluble non-protein nitrogen and 66.9 per cent. as much alpha-amino nitrogen as was removed with distilled water at a temperature of 25° C.

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GROWTH, ORGANIC NITROGEN FRACTIONS, AND BUFFER CAPACITY IN RELATION TO HARDINESS OF PLANTS[1]

S. T. Dexter[2]

Introduction

Since the evidence in the literature has become somewhat confused upon several points relating to winter hardiness in plants, it seemed desirable to investigate some of the points of controversy according to a newer technique and with the benefit of more specific information that has recently become available. The newer emphasis, that hardiness does not exist as such in a hardy variety, but that it must be developed, has been the result of the work of a number of investigators. Within the past year or two it has been shown that winter wheat plants of the most hardy varieties harden only poorly when placed at a low temperature in the dark (**15, 3**). The whole matter of increase in resistance to cold has been connected with opportunity for photosynthesis, storage of organic foods, low respiration, and slight vegetative growth (**3, 7**).

Experimentation

In the first experiment it seemed desirable to know whether winter wheat plants added materially to their dry-matter content if stored in a cold-room with artificial illumination. Minhardi plants were grown to an age of three weeks in the greenhouse, after which a part of the crop was moved to a room at 2° C.

In all experiments reported in this paper the plants were grown in quartz sand cultures with nutrient solutions. Six pots with 25 plants each were harvested prior to cold-room treatment, six were stored in the dark at 2° C., and six were stored in continuous light at 2° C. After two weeks the plants in the cold-room were harvested. The hardiness of the plants was determined before and after storage by the freezing-exosmosis method of Dexter *et al.* (**6**). Table I shows the results of this experiment. Since the pots were very uniform, only totals are given.

From the table it would appear that the plants made marked growth, as seen by the more than doubling of the weight of the dry matter contained in them. It was not possible, however, to distinguish the two sets visually after removal from the cold-room. Increase in the length of the leaves was hardly more than the experimental error. The plants that were stored in the light in the cold-room were materially lower in total nitrogen per

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TABLE I

GROWTH OF WINTER WHEAT (MINHARDI) AT 2° C. SHOWING CHANGES IN HARDINESS WHEN ILLUMINATED AND WHEN STORED IN DARKNESS

SAMPLE	TOPS				ROOTS		TOTAL DRY WT.	FREEZING INJURY. SPECIFIC CONDUCTIVITY × 10^6, 25° C.
	DRY MATTER	GREEN WT.	DRY WT.	TOTAL NITROGEN	GREEN WT.	DRY WT. LESS ASH		
	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
At the start	14.0	51.7	7.2	0.218	42.4	4.8	12.0	99.1
14 days 2° C. dark . .	14.1	55.3	7.8	0.267	40.7	4.7	12.5	98.5
14 days 2° C. light	22.9	74.3	17.1	0.444	54.8	9.1	26.2	40.0

gram dry matter than the other sets, although the total nitrogen per plant was much greater in them. It is evident that the plants which received illumination carried on active photosynthesis and took up nitrogen from the soil at the temperature of 2° C. These plants hardened materially, whereas the hardening in the plants stored in the dark was hardly perceptible. Analysis showed that the content of reducing sugars in the plants stored in the dark had dropped to a mere trace during the period in the cold-room.

In the next experiment, winter wheat plants were grown for two weeks in the greenhouse with a full nutrient solution. At the end of that time the nitrogen was washed from half the pots, and the plants were allowed to grow for two weeks without nitrogen in the nutrient medium. Calcium chloride was substituted for calcium nitrate. At the end of two weeks without nitrogen, extracts from the minus-nitrogen plants gave no test for nitrates with diphenylamine and the plants were presumed to be virtually free from inorganic nitrogen. They showed every evidence of nitrogen deficiency.

By means of this experiment it was hoped that several points might be clarified: (1) Does the commonly reported increase in soluble organic nitrogen result from uptake from the soil, or is it due to breakdown of proteins? (2) Does the winter wheat plant require nitrogen in the soil during hardening? (3) Will minus-nitrogen winter wheat plants harden at a low temperature in the dark?

The organic nitrogen fractions in the plants were determined in the following way. Fresh samples (20 plants) were treated with ether and

ground to a smooth paste with quartz sand. The sample was made to a definite volume with tenth normal potassium sulphate, centrifuged, and the residue washed and centrifuged three times. The resulting suspension was coagulated with heat, after adding a few drops of acetic acid. The coagulated material was removed by filtration. The three fractions were determined separately, *i.e.,* the soluble or non-coagulable fraction, the coagulable fraction, and the fraction thrown out by the centrifuge. It was not found possible to obtain checks on duplicates for the latter two determinations, although the "soluble" fraction gave good checks. The "soluble" nitrogen is reported, then, as the percentage of the total nitrogen in the plants. On this basis the duplicate samples checked well. Table II gives the results of this experiment. The experiment was repeated, with the additional analyses for amino nitrogen (Van Slyke), and for carbohydrates. Table II includes these figures. According to a similar idea, samples of cabbage leaves were prepared from plus-nitrogen plants, deficient in starch and actively growing, and from minus-nitrogen plants high in starch. Samples to be compared in any regard consisted of half-leaves, split at the midrib. One set was analyzed as it came from the greenhouse, the other after storage in a moist chamber at 2° C. in the dark for 7 days.

Table II appears to answer the first question as to the increase in soluble organic nitrogen in plants stored at a low temperature. Several workers have noted this increase and have attributed the increase in hardiness partly to this cause (**10, 6**). In a recent paper NEWTON *et al.* (**12**) concludes that the increase in soluble nitrogen, especially in alpha amino form, is due to the occasional freezing of the plants during the early winter season. The plants in this experiment were not frozen, however. That the increase in soluble nitrogen comes from the breakdown of proteins seems probable from the figures presented in the table. Plants which gave no tests for nitrate nitrogen increased as much in soluble nitrogen as those liberally supplied with the element in nutrient solutions, and in which liberal amounts were present, according to the diphenylamine test.

Table II presents the data relating to hardening of these plants at 2° C. both in light and in darkness. Since it was anticipated that the samples might differ greatly in total soluble extractable electrolytes, samples were killed by heating and extracted to give the total salts in the same volume of water as was used for the freezing-exosmosis test (**4**). The samples of winter wheat crowns were frozen at −8° C. for two hours; the cabbage at −6° C. for two hours. The table shows that winter wheat plants high in carbohydrates, due to nitrogen starvation, hardened well in the cold room. Those in the light hardened more than those in the dark, but in either case mineral nitrogen did not seem to be necessary for the hardening process. High-carbohydrate plants of either wheat or cabbage hardened well in the

TABLE II

ORGANIC NITROGEN FRACTIONS IN PLUS- AND MINUS-NITROGEN PLANTS BEFORE AND AFTER EXPOSURE TO LOW TEMPERATURE (2° C.)

SAMPLE*	DAYS AT 2° C.	ILLUMINATION	PERCENTAGE N IN SOLUBLE FORM	PERCENTAGE N IN AMINO FORM	CARBOHYDRATES AS PERCENTAGE DRY MATTER			SPECIFIC CONDUCTIVITY (× 10^6, 25° C.) AFTER	
					REDUCING	SUCROSE	STARCH	FREEZING INJURY	HEATING (TOTAL SALT)
			%	%	%	%	%		
Winter wheat									
Plus-N	0	...	25.3					75.7	116.9
	5	Light	18.3					48.5	116.5
		Dark	28.6					72.5	116.2
	10	Light	26.2					31.1	97.2
		Dark	31.9					69.1	115.0
Minus-N	0	...	21.1					65.1	103.5
	5	Light	22.1					33.6	85.7
		Dark	26.9					49.7	98.7
	10	Light	24.3					25.6	83.8
		Dark	30.7					39.6	92.8
Repeated									
Plus-N	0	...	20.0	9.94				...	...
	10	Light	29.1	17.9					
		Dark	33.1	14.2				...	
Minus-N	0	...	19.7	4.98	1.85	4.8	5.42	52.8	88.2
	10	Light	22.5	10.60	2.91	20.55	12.26	22.5	75.3
		Dark	30.0	16.95	3.88	11.12	3.41	29.9	85.8
Cabbage					Total sugars				
Minus-N	0		8.80		22.2			67.4	181
	7	Dark	17.46		29.0			37.4	160
Plus-N	0	...	13.96		No test for starch qualitatively			126.5	227
	7	Dark	19.48					133.6	223

* Part of the winter wheat samples were stored at 20° C. with continuous illumination, part in the dark. All cabbage samples were stored in the dark. Conductivity values for exosmosis from wheat crowns and from cabbage leaves following freezing injury and following heating are included.

dark; low carbohydrate plants little or not at all. If illuminated, plus-nitrogen plants hardened well.

To summarize the results of this experiment it may be stated that: (1) Proteins are split in the plants stored at this low temperature entirely regardless of the hardening process. In every case the plants which hardened least increased most in soluble nitrogen. (2) Cabbage and winter wheat plants do not require nitrogen from outside the plant to carry on the hardening process. (3) Minus-nitrogen plants, high in starch, hardened well at 2° C. without illumination, in sharp contrast with those low in carbohydrates.

Certain elements were not included in the nutrient solutions in the sand cultures. The salts used were calcium nitrate, potassium acid phosphate, and magnesium sulphate, with traces of iron as phosphate. Tests for chlorides in the extracts from the crowns showed mere traces. Seemingly chlorides are not necessary in the hardening process but did not prevent it, since calcium chloride was used in the minus-nitrogen series. The apparent loss of minerals from the samples of winter wheat which harden has been described more fully in another paper (**4**).

It seemed desirable to investigate the hardening capacity of other minus-nitrogen plants. Winter rye, winter barley, and winter oats were grown as previously described. As in the case of winter wheat, minus-nitrogen plants of rye, barley, and oats were found to harden in the dark at 2° C., whereas plus-nitrogen plants hardened very slightly. To a few pots of plus-nitrogen plants of these four species, a 2 per cent. sucrose solution was added, and the plants set at 2° C. in the dark. With all four cereals hardening was somewhat better than the corresponding plants which did not receive sugar, but not so complete as the minus-nitrogen plants in the dark (**2**). According to a similar idea, the freshly severed stems of cabbage plants were placed in water, in 2.5 and 10 per cent. sucrose solutions, and kept in the greenhouse for 40 hours. The samples were then analyzed for sugars, for freezing injury at − 6° C., and for ice formed, by the calorimetric method. The results were perfectly regular and orderly. More water was left unfrozen in the plants placed in 10 per cent. sugar than in 2.5 per cent., which in turn showed more unfrozen water than the plants kept in water alone. Average water unfrozen in four samples of each was 9.95, 8.70, and 6.94 gm. in the 10 per cent. sucrose, the 2.5 per cent. sucrose, and the water only, respectively. Injuries, according to specific conductivities taken, were 44.5, 59.5, and 75.1 for the samples as just given. The amount of total sugars in the plants kept in 10 per cent. sugar was more than double that in the others. Peculiarly enough the reducing sugars were about twice as great in amount as in the normal plants, although sucrose only was supplied. Evidently the plant has a capacity to hydrolyze rapidly

sugars furnished in this way. The marked protection against freezing injury might well be explained by the decrease in the ice formed. The increase in hardiness, however, was far less than can readily be accomplished by simple exposure to low temperatures for a week, during which time the increase in sugar is far less than was found in these cases. It seems improbable that increase in hardiness can usually be explained in such simple terms.

In another experiment, the buffer capacity of samples of cabbage leaves before and after hardening was investigated. Half-leaf samples were again prepared from plants high and low in carbohydrates respectively. One set of half-leaves was ground with a pinch of quartz sand before exposure to cold; the other half-leaves were put to harden in the cold room for seven days. They were stored in a moist chamber in the dark. After grinding to a smooth paste, the samples were made to a definite volume, and the hydrogen ion concentration determined with a hydrogen electrode. Definite volumes of tenth normal hydrochloric acid or sodium hydroxide were added with a pipette to half the sample, and the hydrogen ion concentration again determined, until five additions of acid or base had been made. A total of eight samples of each type of sample was run, on two occasions. The voluminous data obtained will not be presented in full. In seven out of eight samples of cabbage leaves which actually hardened at 2° C., the ground leaves were found to be slightly more alkaline after exposure to cold than before; in six out of eight samples of low carbohydrate leaves, which did not actually harden at 2° C., the leaves were found to be slightly more alkaline after exposure to cold than before. Thus whether the plants hardened or failed to harden, this response appears to be more or less identical. The changes were practically the same in each case and amounted to about 0.2 pH unit. In all cases, however, the plants that were high in carbohydrates were distinctly more acid than those low in carbohydrates. Those high in nitrogen were found to be better buffers, as well, either before or after exposure to cold. Exposure to cold did not appear to change the buffer capacity in either case.

The increase in hardiness has been attributed to an increase in soluble nitrogen and an increase in buffer capacity. An increase in soluble nitrogen unquestionably occurs on exposure to low temperatures, but it occurs to a greater degree in plants that have not hardened than in those which have. NEWTON (**11**) failed to find a correlation between buffer capacity and hardiness in winter wheat. HARVEY (**8**), who reported a definite increase in buffer capacity in cabbage which was hardened, used the expressed juice from the plants. This expressed juice, after hardening, is usually higher in total solids than before hardening, and might well show more buffer capacity since it contains more dry matter. In the technique

used in this experiment, however, virtually the same amount of dry matter was used in each case. No change in buffer capacity was found in a given number of cells or a given amount of material. The increase in soluble nitrogen in high carbohydrate plants during exposure to cold is not particularly great, since the quantities present are small. In the samples analyzed, less than 0.3 mg. of soluble nitrogen was present per gram of fresh leaves. Even though this amount almost doubles during exposure to cold, it must still be a very small factor in the buffering of the plant juice. The amount of soluble nitrogen present in the vegetatively active cabbage plants was about four times this amount.

In view of the findings of these previous experiments, it seemed desirable to reinvestigate the matter of the hardening of plants in an alternating

TABLE III

CHANGE IN HARDINESS, SOLUBLE NITROGEN, AND SUGARS IN HALF-LEAF SAMPLES OF CABBAGE STORED FOR SEVEN DAYS IN THE DARK AT CONSTANT LOW, ALTERNATING HIGH-LOW, AND INTERMEDIATE TEMPERATURES

Sample	Freezing injury. Specific conductivity × 10^{-5}			Soluble N		Sugars					
						Reducing			Sucrose		
	2°	2°–20°	15°	2°	2°–20°	2° C.	2° C.–20° C.	15° C.	2° C.	2° C.–20° C.	15° C.
	°*C.*	°*C.*	°*C.*	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	236	595	621								
2	172	462	508								
3	140	281	264								
4	271	552	604								
5	419	419	286								
Average	247	462	457	15.1	41.1	0.613	0.543	0.549	0.272	0.140	0.099

temperature. HARVEY (9), TYSDAL (16), and others in unpublished work, have observed increased hardening in plants that were subjected to alternating temperatures. Frequently these plants were given continuous illumination, whether at a constant or an alternating temperature. DEXTER (3) and TYSDAL (16) have used various lengths of day and night during hardening. DEXTER has presented data which seem to indicate that the increased hardening under such conditions is due to increased photosynthesis and greater net storage of organic foods. In the experiment now reported, half-leaf samples were stored at 2° C. continuously. The other halves were alternated between 2° and 20° C. at approximately 12-hour intervals. A third sample, as nearly like the duplicate half-leaf samples

as possible, was stored at a constant temperature of 15° C. It was estimated that the respiration of this sample would be approximately that of the sample alternated between 2° and 20° C. The samples were contained in moist chambers, in the dark, and no wilting was evident at the end of the experiment although those at the higher temperatures were somewhat yellowed. Five samples were stored under each temperature condition for examination of hardiness. Samples were also prepared for examination of change in soluble nitrogen and for sugar analysis. Table III presents the data from this experiment.

From the data given in table III, it can be seen that the samples stored at 2° C. hardened a great deal more than duplicate half-leaf samples alternated from 2° to 20° C. Each set was in the dark. The samples alternated appear to be almost precisely as hardy as the samples kept constantly at 15° C. These were not duplicate half-leaf samples but were as nearly identical as possible. The soluble nitrogen in the leaves kept at 2° C. continuously is less than half that of the alternated leaves. The increase in soluble nitrogen in the alternated leaves evidently did not give them greater hardiness. The sugars, and especially sucrose, were higher in the sample kept continuously at 2° C. than in either of the other two samples. The other two samples agree perhaps as well as could be expected, since they were not duplicate half-leaves. These data would seem to support the idea previously put forth (3) that if greater hardening is found under conditions of alternating temperature, above the freezing point, it is due to the greater photosynthesis under those conditions. Alternating temperatures, with the plants in the dark, or in the light without carbon dioxide, do not seem to stimulate the hardening reaction.

Summary and conclusions

1. A reexamination of some of the theories of winter hardiness of plants has been made. It should be recognized in any work dealing with the hardening reaction, that plants which are usually thought of as hardy need not necessarily harden if placed at a low temperature. This reaction seems to be largely dependent upon the organic nutrition of the plant, if opportunity for photosynthesis is denied at the low temperature.

2. Plants of a hardy variety of winter wheat were grown with excess and with minimum nitrogen supplies in the nutrient medium. When placed at 2° C. without illumination, it was found that those high in nitrogen did not harden, although there was a considerable increase in soluble organic nitrogen in the plants during the period at the low temperature. The minus-nitrogen, high-carbohydrate plants hardened well in the dark with an increase in soluble organic nitrogen. Sugars increased in the high-carbohydrate plants and decreased in the plus-nitrogen plants during

such storage. Although the behavior in regard to organic nitrogen was almost indistinguishable, the difference in hardening was marked. With both sets of plants in the light at a low temperature, hardening was efficiently carried out, and the increases in soluble organic nitrogen were less than with corresponding plants in the dark, where hardening was in all cases less. Thus increase in soluble organic nitrogen is no indication of increase in hardiness.

3. Minus-nitrogen, high-carbohydrate plants of winter rye, winter barley, winter oats, and cabbage were found to harden at 2° C. in the dark, while corresponding plants low in carbohydrates due to surplus nitrogen in the nutrient medium did not harden under those conditions.

4. Cabbage leaves, low in carbohydrates and high in nitrogen, although they hardened but little at a low temperature were found to be more alkaline and better buffers, both before and after exposure to cold, than leaves high in carbohydrates and low in nitrogen. While the samples of both sets were generally slightly more alkaline after exposure to cold for a week, they were not distinguishably better in buffering capacity than before exposure to cold.

5. Constant low temperature in the dark was more effective in causing hardening in cabbage leaves than alternating temperatures in the dark. Higher sugar content was found in the samples kept constantly at 2° than in samples alternated between 2° and 20° or stored at a constant temperature of 15° C. Soluble nitrogen was higher in the alternated leaves than in those held at 2° C.

6. There appears to be a series of reactions which proceed in a plant at a low temperature entirely regardless of increase in hardiness. There is an increase in soluble organic nitrogen. There is a decrease in respiratory rate which is not correlated with increase or decrease in sugars or enzyme activity (**5**, **1**). There is, usually at least, an increase in sugars, which may or may not be accompanied by decidedly increased hardiness. A continued and critical examination of the theories of winter hardiness is needed.

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RELATIONSHIP BETWEEN NITROGEN FERTILIZATION AND CHLOROPHYLL CONTENT IN PINEAPPLE PLANTS[1]

R. K. TAM AND O. C. MAGISTAD

(WITH TWO FIGURES)

Introduction

Since nitrogen is an essential part of the chlorophyll molecule, any appreciable lack of it in the mineral nutrient supply of the plant should result in a suppression of chlorophyll formation. The extent of this suppression would, of course, be modified if there were a deficiency in other growth factors such as light, iron, magnesium, carbon dioxide, and water.

It is the purpose of this paper to show the relationship existing in leaves of pineapple plants between the total chlorophyll ($\alpha + \beta$) concentration and various amounts of nitrogen applied as fertilizer in a series of field experiments.

According to SCHERTZ (8), VILLE in 1889 was probably the first investigator to note a definite relationship between nitrogen supply and chlorophyll formation. The color of the plants observed varied according to the amount of nitrogen applied. Similar observations were made by PINCKNEY (5) in the case of sorghum fertilized with sodium nitrate.

SIDERIS (unpublished data) has shown that leaves of pineapple plants receiving very large quantities of nitrogen had an increased content of amino acid and chlorophyll.

SCHERTZ (6), working with *Coleus blumei* (var. Golden Bedder), found that the mottling of leaves was due to a lack of nitrogen and could be prevented by the addition of sodium nitrate. The addition of iron, magnesium, calcium, or phosphate failed to prevent mottling and subsequent shedding of the leaves by the plant. On the other hand, BRIGGS, JENSEN, and McLANE (1) were unable to establish a relationship between the percentage of leaves mottled and the total nitrogen content in the soil in both the orange and lemon groves studied. In a later investigation SCHERTZ (8) concluded that the amount of nitrogen supplied correlated with the amount of chloroplast pigments present in the fresh green leaves of cotton and potato plants.

ULVIN (10) recently found that more chlorophyll was produced by plants of sugar cane receiving nitrogen as nitrate than by those receiving nitrogen as ammonia at each of three soil temperatures. The application of 1 to 15 p.p.m. of iron caused a marked increase in chlorophyll formation.

[1] Published with the approval of the Director as Technical Paper no. 77 of the Pineapple Experiment Station, University of Hawaii.

GUTHRIE (3) showed that the availability of nitrates affected the chlorophyll content of plants differently during winter and spring. The lack of nitrates in winter did not induce any chlorophyll loss, but in spring a great reduction occurred when there was an insufficient supply of nitrates.

Experimentation

Material

Total chlorophyll ($\alpha+\beta$) determinations were made on pineapple leaves from four field experiments. Table I gives the location, number of plants per acre, and fertilization rate in pounds of nitrogen per 1000 plants for each of the treatments in the four experiments. The amount of nitrogen indicated was applied during the growing period of approximately 20 months.

TABLE I

DESCRIPTION OF FIELD EXPERIMENTS FROM WHICH LEAF MATERIAL WAS OBTAINED

EXPERIMENT NO.	LOCATION	PLANTS PER ACRE	FERTILIZATION RATE IN LB. N PER 1000 PLANTS
I	Field 6, Kunia	15,600	0, 6.4, 12.8, 19.2, 25.6, 38.4
II	Field 4719, Opaeula	12,300	0, 8.1, 16.2, 24.3, 32.4, 48.6
III	Field 15a, Wahiawa Substation	9,680	0, 10, 20, 30, 40, 60
IV	Field 45, Maui	17,424	0, 5.7, 25.8, 37.2, 42.9, 48.6

Experiments I, II, and III were each divided into two parts. Part A received its nitrogen in two applications, at the time of planting and at the age of 12 months. Part B received its nitrogen in four applications, one-fourth at the time of planting and equal amounts at the age of 6, 12, and 18 months. The leaf samples were obtained shortly prior to harvest when the pineapple fruits were still green but nearly full size.

All four experiments were installed in the fall (September and October) of 1932. They were regularly and well supplied with iron as ferrous sulphate spray.

Ammonium sulphate was used as the nitrogen source in all four experiments. Neither potassium nor phosphorus was applied in experiments I, II, and III. All of the plots in experiment IV had been uniformly fertilized with 5.8 lb. P_2O_5 and 2.9 lb. K_2O per 1000 plants.

Method of determination

The method used in the extraction and separation of chlorophyll from the other plant pigments was that of WILLSTÄTTER and STOLL (11) as

modified by SCHERTZ (7). It was found after a few trials that the great variation in chlorophyll content of plants between treatments made it necessary to vary the size of the samples taken for chemical analysis. Accordingly, 25 gm. were used in the case of the samples from the low nitrogen plots while 15 and 10 gm. samples were used for the plants fertilized with larger amounts of nitrogen. Care was taken to grind the larger samples for a longer period of time than the smaller samples in order to obtain an equal degree of extraction.

Subsequent to extraction, saponification, and separation, the chlorophyllins were run into a 100-cc. volumetric flask and made to volume with distilled water. Determinations were made by comparison, in a Klett colorimeter, with the artificial color standard developed by GUTHRIE (2).

SAMPLING

Before proceeding with the regular analyses of the samples from the different experiments, it was desired to determine the variations existing in the chlorophyll content of plants within the same plot. A knowledge of the degree of the variations would provide information as to the number of leaves to use for a reliable sample.

Single leaf samples from each of twelve separate plants were obtained from the plot receiving 25.6 lb. nitrogen per 1000 plants of experiment IA. Total chlorophyll determinations were made on these twelve samples, and the means, standard deviations, and coefficients of variability were calculated. These appear in table II.

TABLE II

VARIATION IN CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS WITHIN A SINGLE PLOT

STATISTIC	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
	mg.	*mg.*	*mg.*
Mean	61.36	336.65	13.71
Standard deviation, σ_x	± 5.22	± 31.17	± 1.20
Coefficient variability, C. V.	8.51	9.26	8.75

From a knowledge of the standard deviation of these twelve samples (table II) and the great range in chlorophyll content at various nitrogen levels, it was decided that a sample consisting of one leaf from each of three separate plants, within the same plot, would constitute a sufficiently reliable analysis. Accordingly all subsequent samples were composited from three

leaves. The longest leaves were used and fall in group L–III (9). At the time of sampling all plants were approximately 20 months old with fruits nearing maturity.

Determination of leaf area

In order to calculate chlorophyll content per unit area, some means of measuring leaf areas was necessary.

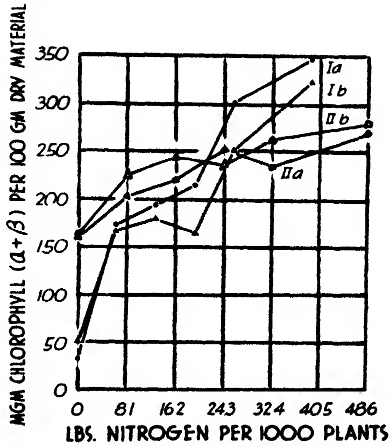

Fig. 1. Chlorophyll (α + β) content of pineapple leaves with varying amounts of nitrogen applied; dry weight basis.

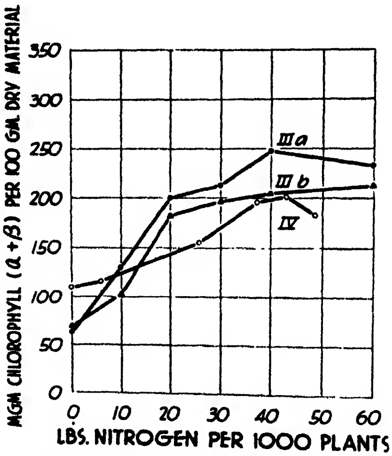

Fig. 2. Chlorophyll (α + β) content of pineapple leaves with varying amounts of nitrogen applied; dry weight basis.

Krauss (4), in studies on the transpiration of pineapple plants, determined the area (upper and lower) of large pineapple leaves by considering them triangles of small base and great altitude, and used the formula: area = length × width.

The width was measured along an imaginary line near the base of the leaf at the junction of white and green tissue. The length was taken from this line to the tip of the leaf.

This formula was used in the present study to determine the area, except that the product of the length times the width was multiplied by 0.5 to obtain the area from one side of the leaf only. The widths were taken 5 cm. from the base of the leaf and the lengths from this point to the tip of the leaf. The formula was checked by a series of planimeter readings of traces of representative leaves. Calculated on the basis of eleven readings, a mean

TABLE III

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT I

	N PER 1000 PLANTS	MOISTURE	AREA FOR 100 GM. WET BASIS	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
	lb.	*%*	*sq. cm.*	*mg.*	*mg.*	*mg.*
I A	0	80.67	633.33	6.4	33.09	1.02
	6.4	82.61	601.11	30.3	174.23	5.04
	12.8	81.29	530.73	36.5	194.91	6.88
	19.2	81.40	537.44	40.0	215.20	7.44
	25.6	80.69	509.81	58.0	300.44	11.38
	38.4	81.60	477.91	64.0	347.52	13.40
I B	0	81.22	513.27	9.4	50.01	1.84
	6.4	81.35	633.99	31.3	167.77	4.94
	12.8	81.34	458.27	33.5	179.56	7.30
	19.2	81.07	516.84	32.0	168.96	6.20
	25.6	80.41	488.65	49.1	250.16	10.02
	38.4	82.14	488.98	58.0	324.80	11.62

deviation of 3.16 per cent. was obtained between actual planimeter readings and values obtained through the use of this formula.

After discarding the basal 5 cm. of white tissue, the total wet weights of the three leaf samples were recorded. The leaves were then ground in a meat chopper and their total moisture determined. The total chlorophyll determinations were begun immediately after the samples were ground.

RESULTS

The results of the determinations of the chlorophyll content of the samples from the four field experiments are found in tables III, IV, V, and

VI. Chlorophyll concentrations have again been calculated on both the wet and dry weight bases and also on the 100 sq. cm. wet basis.

Figures 1 and 2 show the graphic relationship existing between the amount of nitrogen applied and the chlorophyll concentration of plants from the various plots in the four experiments. Chlorophyll values obtained on the wet weight basis were used in the construction of these curves.

TABLE IV

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT II

	N PER 1000 PLANTS	MOISTURE	AREA FOR 100 GM. WET BASIS	CHLOROPHYLL 100 GM. WET BASIS	CHLOROPHYLL 100 GM. DRY BASIS	CHLOROPHYLL 100 SQ. CM.
	lb.	%	*sq. cm.*	*mg.*	*mg.*	*mg.*
IIA	0	83.57	525.78	26.57	161.81	5.06
	8.1	84.71	567.92	31.10	203.39	5.48
	16.2	82.94	550.54	37.50	219.75	6.82
	24.3	82.49	526.55	43.97	251.07	8.36
	32.4	82.50	496.81	41.13	234.85	8.28
	48.6	81.77	515.38	49.04	269.23	9.52
IIB	0	83.15	606.25	27.72	164.38	4.58
	8.1	83.37	543.02	37.50	225.38	6.90
	16.2	83.68	481.59	39.85	244.28	8.28
	24.3	82.59	534.03	41.13	236.09	7.70
	32.4	82.07	534.70	47.22	263.49	8.84
	48.6	80.98	499.65	53.13	279.46	10.64

Discussion

Natural variation in concentration of chlorophyll in leaves from a single plot is appreciable, as shown in table II. On the weight basis, the standard deviation of a determination was $\pm$ 5.22 with a coefficient of variability of 8.51 per cent. This coefficient of variability was the lowest of the three that were obtained on the different bases. Obviously, variations in moisture contents and discrepancies between weights and areas contributed to the higher values obtained on the dry weight basis and on the area basis.

Dividing the standard deviation of $\pm$ 5.22 by $\sqrt{3}$, a value of $\pm$ 3.01 is obtained. This is the standard error of a determination using a composite sample of three leaves, one each from separate plants within a plot. With

TABLE V

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT III

	N PER 1000 PLANTS	MOISTURE	AREA FOR 100 GM. WET BASIS	CHLORO-PHYLL 100 GM. WET BASIS	CHLORO-PHYLL 100 GM. DRY BASIS	CHLORO-PHYLL 100 SQ. CM.
	lb.	%	*sq. cm.*	*mg.*	*mg.*	*mg.*
IIIA	0	83.57	473.37	10.62	64.68	2.24
	10	82.90	473.19	22.14	129.52	4.68
	20	82.38	442.00	35.40	201.07	8.00
	30	82.54	460.52	37.50	214.88	8.14
	40	81.69	545.28	45.55	248.70	8.36
	60	80.54	523.91	45.55	234.13	8.70
IIIB	0	84.05	449.22	11.08	69.47	2.48
	10	82.68	501.37	17.68	102.01	3.52
	20	82.64	476.20	31.90	183.74	6.70
	30	82.56	458.29	34.45	197.40	7.52
	40	81.77	491.36	37.50	205.88	7.64
	60	81.33	494.68	59.85	213.60	8.06

TABLE VI

CHLOROPHYLL CONTENT OF PINEAPPLE PLANTS RECEIVING VARYING NITROGEN FERTILIZATION: EXPERIMENT IV

N PER 1000 PLANTS	MOISTURE	AREA FOR 100 GM. WET BASIS	CHLORO-PHYLL 100 GM. WET BASIS	CHLORO-PHYLL 100 GM. DRY BASIS	CHLORO-PHYLL 100 SQ. CM.
lb.	%	*sq. cm.*	*mg.*	*mg.*	*mg.*
0	81.26	578.66	20.71	110.59	3.58
5.7	81.23	633.49	21.78	116.09	3.44
25.8	81.16	558.79	29.27	155.42	5.24
37.2	81.41	582.48	36.45	196.10	6.26
42.9	81.40	562.17	37.50	201.75	6.68
48.6	81.25	544.82	34.45	183.62	6.32

large differences in chlorophyll content for the various treatments, the sampling error becomes relatively small.

Figures 1 and 2 show graphically the relationship existing between the increase in chlorophyll content and the increase in nitrogen fertilization. Increases were noted in all four experiments. In general, with every increase in the amount of nitrogen applied a corresponding increase in the chlorophyll concentration was noted. There were slight deviations from the rule, however. These exceptions were experiment IB with the 19.2 lb. per 1000 plants application; IIA with the 32.4 lb. application; and IIB with the 24.3 lb. application. A decrease in the chlorophyll content of the plants of the plot fertilized with the highest amount of nitrogen occurs in experiment IIIA, and may possibly be due to a state of excessive nitrogen fertilization.

The chlorophyll concentrations of the plants receiving no nitrogen in the four experiments varied considerably. Experiments I and III showed the lowest chlorophyll concentrations, while samples from experiment II contained the highest amount of chlorophyll of the four experiments. These different levels of chlorophyll concentration would seem to be correlated with the differences in nitrogen availability between the areas in which the experiments were located. However, analyses of soils from these plots for available nitrogen were not made.

The greatest response, from the standpoint of chlorophyll increase, was recorded in experiment IA. The range, from the plot receiving no nitrogen to that receiving 38.4 lb. per 1000 plants, was from 33.09 to 347.52 mg. of chlorophyll per 100 gm. dry material. The increase was more than tenfold. Field observations of the plots in this experiment indicated such a great increase to be possible. The plants in the plots receiving no nitrogen were decidedly chlorotic and underdeveloped, while those receiving the high nitrogen applications were large, dark green in color, and extremely healthy. Fruiting in the former case was also conspicuously retarded. Incidentally, samples from the plot with no nitrogen applied in this experiment gave the lowest chlorophyll concentrations of all four experiments.

Experiment III also showed a good response to nitrogen fertilization. Although the maximum amount of nitrogen applied was greater than in experiment I, the total chlorophyll concentration was not so great.

The least response to nitrogen fertilization was noted in experiments II and IV. The chlorophyll levels in the plots receiving no nitrogen were high in both experiments. In both cases the total chlorophyll concentrations of the plants from the plots receiving the highest amount of nitrogen were less than twice that of the plants from the plots receiving no nitrogen.

The number of applications of nitrogen had little effect on chlorophyll content. Except for experiment II, the chlorophyll determinations of the

plants receiving the nitrogen in two applications (A) showed slightly higher chlorophyll concentrations. This was most probably due to the fact that the plants in these plots received their full quota of nitrogen sooner than the plants receiving it in four applications (B). Applying the nitrogen at an earlier period allowed this difference in nitrogen availability between early and late applications to become a factor in chlorophyll formation up to the time of the analysis. The small response in chlorophyll formation due to nitrogen fertilization in experiment II was probably the cause for this exception.

Summary

Leaf samples from four field experiments, fertilized with varying amounts of nitrogen, were analyzed for their total chlorophyll ($\alpha + \beta$) concentrations. Variations in the total concentrations were noted between different experiments and between plots within the same experiment. With few exceptions, an increase in the amount of nitrogen applied resulted in a corresponding increase in the total chlorophyll concentration. This would indicate that, in pineapple plants, the amount of available nitrogen present determines to a large degree the amount of chlorophyll formed, provided the other requirements for chlorophyll formation, such as light, iron supply, and magnesium, are present in sufficient quantities.

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A MECHANISM FOR CONTROLLED CONTINUOUS FLOW OF NUTRIENT SOLUTIONS[1]

F. P. MEHRLICH

(WITH FIVE FIGURES)

Introduction

The composition of a nutrient medium is constantly changed by the plants growing in it. Constant renewal of the nutrients is therefore imperative if the plant condition is to be correlated with a known composition of medium.

The apparatus for continuously renewing nutrient solutions described in this paper is a modification of SHIVE's device.[2] It is essentially a closed system, difficult for dirt to enter, and has a minimum of free surfaces from which evaporation may occur to concentrate the nutrients. While SHIVE's apparatus has a capacity of 2 or 3 liters and requires daily refilling with consequent disturbing of the siphons, the present device holds at least 38 liters and the reserve nutrient may be renewed repeatedly without upsetting the rate of flow. It is practically self starting, more compact and more rugged than other devices, and is suitable for use in exposed places and in the field.

Since it is wholly separate from the plant contained, whatever container is preferable from other considerations may be used. The container may be moved about for examination or manipulation of the plant without interfering with the rate of nutrient delivery. This apparatus may be used with solution, sand, or soil cultures.

Apparatus

The apparatus is composed of six primary parts: (1) inverted reservoirs; (2) constant-level reservoir; (3) tubulatures connecting the two types of reservoirs; (4) air-tubulature adjustment; (5) capillary siphons leading from the constant level reservoir to the plant containers; and (6) capillary-siphon adjustment. Figure 1 illustrates a single complete unit, showing the relationship of the larger parts. The tubulatures and tubulature adjustments are shown in figure 2.

INVERTED RESERVOIRS.—The inverted reservoirs may be of any size or shape desirable for special needs; five-gallon glass carboys are well suited for most purposes. Provision has been made for the use of two or more such reservoirs per unit of the apparatus described in this paper. Thus an

[1] Published with approval of the Director as Technical Paper no. 78 of the Pineapple Experiment Station, University of Hawaii.

[2] ALLISON, R. V., and SHIVE, J. W. Amer. Jour. Bot. 10: 554–567. 1923.

adequate reserve of nutrient solution may always be maintained without appreciably changing the level of the constant-level reservoir. Ordinarily only one carboy is used at a time. When it is nearly empty the second is filled and put into place. The adjustment to the carboys is set so that the first empties completely before the second one is drawn from. This feature,

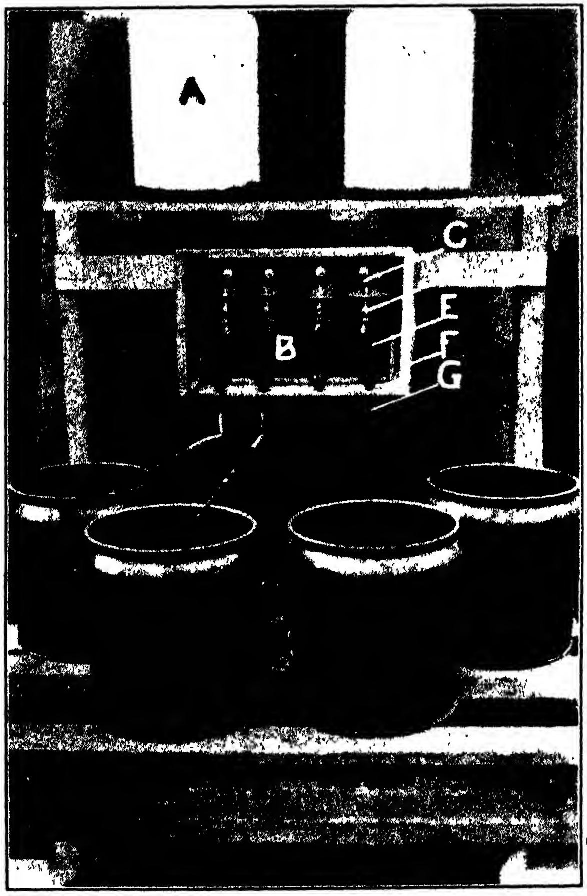

FIG. 1. Single unit of drip apparatus: *A*, inverted reservoir; *B*, constant-level reservoir; *C*, capillary-siphon rough adjustment; *D*, capillary-siphon fine adjustment; *E*, capillary-siphon; *F*, siphon shield; *G*, funnel. The front of the siphon shield and the funnel cover were removed prior to photographing.

however, makes possible the refilling of the two carboys almost simultaneously if special need requires it. Thus a week's supply may be provided at one time. For certain purposes it may seem more desirable to use only one inverted reservoir, or to connect a number of them in trains as described under a separate heading.

The carboys and glass tubulatures are painted first black and then white, to inhibit the growth of algae and to minimize temperature fluctuations.

CONSTANT-LEVEL RESERVOIR.—Tests have shown that the inverted reservoirs (carboys) give out slightly increased quantities of solution as they become progressively empty. Using a small volume constant-level reservoir, such as a glass tumbler, fluctuations in level as great as 2 cm. may occur, upsetting the rate of flow to the plant containers. A large surface reservoir such as that pictured in figures 1 and 2 minimizes this fluctuation. Glass trays or graniteware pans 12 inches by 8½ inches in size are recommended.

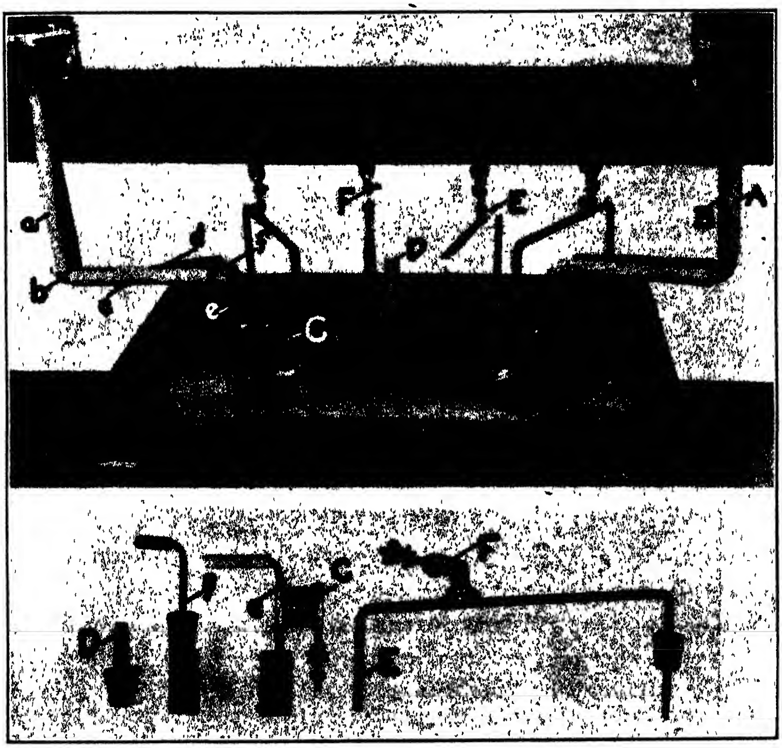

FIG. 2. Constant-level reservoir and fittings: *A*, air channel between inverted reservoir and constant-level reservoir; *B*, solution channel between the two types of reservoirs; *C*, air-tubulature adjustment; *D*, vent tube; *E*, capillary-siphon; *F*, capillary-siphon fine adjustment; *a–e*, various sections of air channel; *f*, final section of solution channel.

Their use reduces the maximum fluctuation to 2 mm. A cover of 1½-inch pine wood or ⅝-inch ply wood has been used successfully. A number of holes of suitable diameter to admit a no. 3 rubber stopper are cut in it to accommodate as many capillary siphons as are desired (fig. 2). At each end of the cover three holes are cut, one to accommodate a no. 3 rubber stopper

fitted with the tubulature for carrying solution from the inverted reservoir (fig. 2 *f*). The other two holes are for the adjustment of the air inlet to the inverted reservoir (fig. 2 *e*, *C*). Still another opening is cut in a central position to admit a no. 3 stopper carrying a small glass tube loosely plugged with glass wool (fig. 2 *D*). This tube may be withdrawn for cleaning the apparatus or to make possible a measurement of solution level in the reservoir. It also maintains the system at atmospheric pressure.

After all holes are made, the cover is waterproofed with hot paraffin. The tubulatures are adjusted to the proper depth and the cover is bolted in position to the shelf supporting the pan. A seal of paraffin and beeswax is made between the periphery of the pan and its cover to preclude dirt.

TUBULATURES BETWEEN THE RESERVOIRS.—The tubulatures illustrated in figure 2 serve two functions: they act as channels for air and solution to move between the reservoirs, but they also determine and maintain the solution level in the constant-level reservoir.

Two parallel tubulatures having an inside diameter of not less than 5 mm. are required: the air channel with white rubber tubing (fig. 2 *A*) and the solution channel with red rubber tubing (fig. 2 *B*). Each is divided into a number of sections to increase ease of manipulation while also maintaining immovable the section determining the solution level. The sections, proceeding from the inverted reservoir to the constant-level reservoir, are as follows: The no. 6 rubber stopper closing the inverted carboy is fitted with two pieces of glass tubing, 7 mm. outside diameter, the one protruding into the neck of the bottle 2.5 cm. and the other, a part of the air channel, ending at the inner surface of the stopper. Both tubes extend below the stopper about 2 cm. Permanently attached to these are pieces of rubber tubing approximately 5 cm. long (fig. 2 *a*). The vertical arm of a glass elbow, 7 mm. in diameter and 3 cm. long on each arm, is inserted into the free ends of each of the rubber tubings (fig. 2 *b*). Attaching permanently to the horizontal arm of the elbow on each channel is a length of rubber tubing 2 or 3 cm. long (fig. 2 *c*), coupled to which by a 3-cm. piece of glass tubing is another 8–10 cm. length of rubber tubing (fig. 2 *d*). This latter tubing attaches permanently to the final section of the channels. This final section of the air channel (fig. 2 *e*), which must remain immovable, is of 7-mm. glass tubing (outside diameter) and protrudes 3 cm. into the reservoir. The end of it must be ground at an angle to prevent solution from hanging in it when air should enter.[3] The tubing terminating the solution channel enters through a no. 3 rubber stopper and protrudes approximately 5 cm. into the reservoir (fig. 2 *f*). It is immovably fixed, and the end of it need not be ground. The tubulatures from the left and right inverted reservoirs are the same.

[3] A suitable grinding medium is no. 70 carborundum lubricated at the time of using with turpentine or glycerin.

AIR-TUBULATURE ADJUSTMENT.—A screw adjustment is fixed for slightly raising or lowering the air intake, which in turn increases or decreases the level of solution in the constant-level reservoir as may be required for bringing several units of the apparatus to the same rate of flow. The same adjustment is used to prevent the two carboys from emptying simultaneously. It is composed of a machine screw and pivoting clamp, as shown in figure 2 *C*.

CAPILLARY SIPHONS.—Heavy walled (5 mm. outside diameter) capillary glass tubing having an inside diameter of 1 mm. is desirable for use as siphons to carry solution from the constant-level reservoir to the plant containers. They may be of any length or shape for special purposes. For general use a siphon 8 inches long, bent as illustrated in figure 2 *E*, has served best. The arm extending into the reservoir is 3½ inches long; the free arm is 3 inches long. Siphons of larger bore make the precise adjustment of rate of flow more difficult. The drip from each siphon is caught in a glass funnel (fig. 1 *G*) through which it runs by a larger bore channel (5 mm. inside diameter) to the plant container.

CAPILLARY-SIPHON ADJUSTMENT.—The rate of flow of solution to the plant containers is regulated by raising or lowering the outlet of the capillary siphons. The mechanism for adjusting the inclination of these siphons consists of two units, one for rough adjustment (fig. 3) and the other for

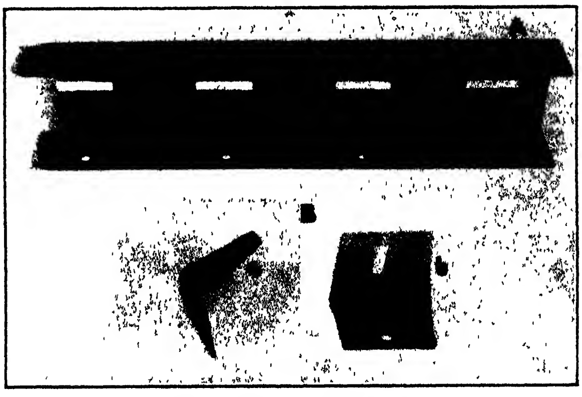

FIG. 3. Capillary-siphon rough adjustment: *A*, *B*, the two primary parts; *a*, side view of part B; *b*, back view of part B.

fine adjustment (fig. 2 *F*).

The rough adjustment consists of two parts as illustrated: (1) a length of heavy sheet metal bent as shown in figure 3 *A*, 14 inches long, 2½ inches high, and 1¾ inches wide. Into it four horizontal slots $\frac{5}{16}$ inch × 1½ inch are cut (if more than four capillary siphons are to be used, a proportional increase in the length of this piece and the number of slots should be made);

(2) a piece of right-angled iron or heavy sheet metal $2\frac{1}{2}$ inches wide (fig. 3 *B*). The vertical and horizontal sides are also approximately $2\frac{1}{2}$ inches. In the vertical face a $\frac{5}{16} \times 1\frac{1}{2}$ inch slot is cut. This face attaches by means of a machine screw to the metal piece just described. Thus both vertical and horizontal movement are possible. When the desired position is attained the screw is tightened and the adjustment remains permanently. In the horizontal face of the right angle piece is a $\frac{5}{16}$ inch hole through which the fine adjustment attaches by means of a machine screw, provided with a winged nut. All screws and nuts are galvanized to prevent rusting. This metal piece may be dispensed with for certain purposes, the angle-iron attaching them to the table directly.

Trains of inverted reservoirs.—Trains of carboys may be set up for cultures in remote places where more frequent renewal of reserves is un-

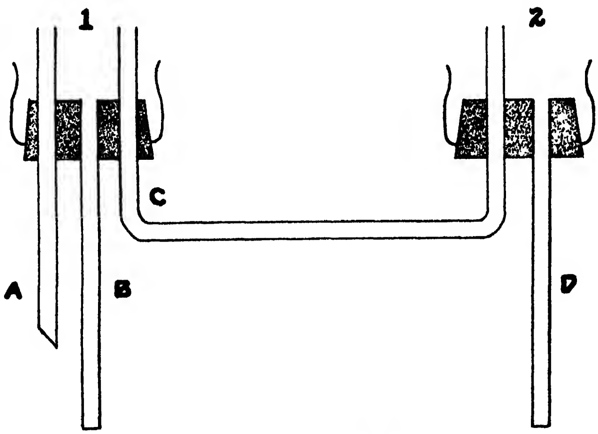

Fig. 4. Tubulature arrangement for connecting inverted carboys in trains: *A*, air channel from inverted reservoir 1 to constant-level reservoir; *B*, *D*, solution channels from inverted reservoirs 1 and 2 respectively to same constant-level reservoir; *C*, air channel connecting inverted reservoirs 1 and 2.

desirable. The connecting tubulatures shown in figure 4 make possible such an increase in the reserve nutriments without disturbing the advantages of the mechanism already described. The same type of connection may be made between the left and right inverted reservoirs as an alternative method to that just described.

Control of algae in sand cultures.—For ordinary purposes the nutrient solution is led into the sand through a blackened glass tube. Clean white quartzite pebbles having a diameter of about $\frac{1}{4}$ inch are spread 2 inches deep over the top of the sand, preventing the growth of algae on the sand surface while at the same time cutting down evaporation.

Precision of apparatus

Table I gives the rates of flow from the capillary siphons of two 4-siphon units for consecutive 24-hour periods, when the rate of flow from each siphon was arbitrarily set at 14 drops per 2 minutes. By manipulating the

TABLE I

Record of drip rate for consecutive 24-hour periods, from two units, each siphon of which is set to deliver 14 drops each 2 minutes*

Day	Unit A				Unit B			
	1	2	3	4	1	2	3	4
	Right side, carboys closed							
1	800	810	840	810	880	890	870	790
2	860	855	885	860	860	870	850	820
3	935	930	935	950	925	935	910	870
	Left side, carboys closed							
4	960	930	930	940	960	930	940	950†
5	969	932	932	960	978	988	969	969
6	969	951	924	969	1006	1015	997	1006
Average delivery	915.5	901.3	907.7	914.8	934.8	938.0	922.7	900.8

* As the carboys empty, increasing amounts of solution are delivered, but all units vary in the same direction.

† Adjustment altered.

adjustments slightly, the drips may be brought into even closer agreement.

As noted in table I, the rate of nutrient delivery increases as the carboys empty, and as the flow changes from the left to the right carboy, but under similar conditions all units vary in the same direction and to the same degree; hence this variation should not enter as a disturbing element in large-scale experiments calling for several units.

Changes of temperature cause fluctuations in flow, as shown in table II, but under the usual range of conditions their magnitude is slight.

Table and plant containers

A table to support the mechanism has been designed for use with pineapple plants (fig. 1). Several units may be combined as a single table to replace the standard planthouse bench. Modifications may be made to adapt this apparatus for use with revolving tables or constant temperature devices.

The position of the siphons is the important factor in controlling the rate of flow of the nutrient solution. The shield (fig. 5) not only protects the siphons from movement or breakage by the plants, but covers the fun-

TABLE II

INFLUENCE OF TEMPERATURE ON RATE OF NUTRIENT SOLUTION DELIVERY SHOWN BY QUANTITIES OF LIQUID DELIVERED IN 2-HOUR PERIODS BY CAPILLARY SIPHONS OF SINGLE DRIP UNIT

TIME	AVERAGE TEMPERATURE*	QUANTITY OF FLOW			
		DRIP 1	DRIP 2	DRIP 3	DRIP 4
	° C.	cc.	cc.	cc.	cc.
8–10 A. M.	27.8	70.0	71.0	70.5	71.5
10–12	30.0	74.0	75.5	76.0	77.0
12– 2 P. M.	30.7	70.0	72.0	72.5	72.5
2– 4	29.3	77.5	80.0	79.0	79.5
4– 6	26.3	71.0	73.0	73.0	72.0
6– 8	24.8	67.5	69.0	68.5	68.0
8–10	23.7	65.5	67.0	66.5	66.0
10–12	23.2	64.0	65.0	65.0	65.0
12– 2 A. M.	22.7	61.0	62.0	62.0	62.0
2– 4	22.7	63.0	64.0	64.0	64.0
4– 6	23.3	64.0	65.5	65.0	65.0
6– 8	24.5	65.0	66.5	66.0	66.0
8–10	27.0	70.0	71.5	71.0	71.0
10–12	28.5	73.0	75.0	74.5	74.5

* Computed thermograph record readings made each 30 minutes.

FIG. 5. Siphon shield for protecting capillary siphons from breakage. The funnels serving as semi-flexible couplings in solution channels are inclosed in the black compartment in lower, forward portion of shield. Cover *A* to the shield and cover *B* to the funnel compartment were moved out of place before photographing.

nels as well, limiting evaporation and the growth of algae. The funnel cover is an opaque compartment painted black on the inside, situated in the lower forward part of the siphon shield as shown in figure 5. The capillary siphons enter the funnels through suitable openings in the top of the funnel cover. Glass wool is used to close the space remaining between each siphon and its opening. The funnels (figs. 1 *G*, 5) serve as a coupling in the solution channel from the capillary siphons to the plant containers, allowing enough movement of the channel to prevent breakage.

Where danger of breakage is not a factor, the shield may be omitted and the siphons increased in length to connect directly with the plant container.

Summary

1. With care, an adjustment of siphons can be made so that variation in amount of solution delivered by a series of units does not exceed 50 cc. per liter; that is, 5 per cent. In view of the low concentrations of nutrient solutions this amount is so small a variation in the actual amount of any one element or compound delivered as to seem inconsequential.

2. More than one rate of drip may be served from a single constant-level reservoir, since rate of flow is determined by the inclination of individual capillary siphons.

3. Rate of flow of nutrients may be altered according to the requirements of the growing plant, without seriously interfering with the study in progress. The siphon easily can be adjusted to deliver more or less nutrient, and the new rate of flow can be measured for a short interval without disturbing the cultured plants.

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GROWTH MODIFICATIONS IN CITRUS SEEDLINGS GROWN FROM X-RAYED SEED

C. P. Haskins and C. N. Moore

(WITH SEVEN FIGURES)

A summary is made in the present paper of certain morphological variations observed in seedlings of the citrus fruits arising from seed exposed to x-rays before planting. The work has been undertaken in the course of a series of investigations on the biological effects of x-rays and cathode rays, and although as yet no cytological examination of the material has been made, it was considered worth while to assemble descriptions of certain morphological effects at the present time, in view of the paucity of x-ray work reported with the citrus fruits, especially at higher voltages and currents. Certain of the variants considered have already been described,[1] but have been included here in order to complete the series.

Seeds of lemon, lime, tangerine, grapefruit, and sweet and sour orange were obtained through the kindness of Dr. R. G. LaRue, Superintendent of Cultivation of the University of California Agricultural Experiment Station, and Dr. A. F. Camp, Head of the Department of Horticulture of the University of Florida. They were dried previous to shipment. A portion of the seed was x-rayed in the condition in which it was received. The remainder, before treatment, was soaked in distilled water for 15 minutes, then left in a completely moisture-saturated atmosphere for 12 hours. The seeds were then dried on filter paper and exposed at once. Treated seeds were planted immediately in seed flats, a mixture of one-third peat moss and two-thirds sharp sand being used, the seeds being covered in most cases with pure sharp sand. The seeds were germinated in an electrically heated greenhouse, day temperatures being maintained constant at 75° F. and night temperatures at 55° F. during the early growth of the plants. At a later time a large number of apparently normal seedlings were transferred to the open nursery, being planted in a sandy loam soil. Most of the more conspicuous variants were maintained in the greenhouse for a full year, however, being transferred to benches containing a mixture of sand, clay, and manure at the age of a few months. After this change they were not disturbed until the close of the experiment.

A Coolidge water-cooled tungsten-target tube of the thick-walled type was used as the source of x-rays, operated at 200 k.v.p. and 30 ma. from a

[1] Science, Aug. 5, 1932.
Science, Feb. 15, 1933.
Bot. Gaz. 94: 801–807. 1933.

high-tension transformer equipped with Snook mechanical rectifier. The seeds were exposed on a lead-covered table at a focal distance of 50 cm. Under these conditions the incident energy input, as measured with a graphite-covered collodion wall, radium-compensated Failla ionization chamber, was 165 roentgens per minute. The shortest wave length theoretically obtainable at this voltage is 0.062 Å. as calculated from the quan-

FIG. 1. Grapefruit seedling flowering prematurely after x-ray treatment of seed from which it came. Dosage 300 r.

tum relation $Ve = hv_{max}$. No metal filter was used, but the glass of the wall interposed a filtering action equivalent to about 0.10 mm. of copper. At 0.70 Å. the intensity of the emergent radiation was only about 0.3 per cent. of that impinging on the inner wall, as calculated from COMPTON's 1926 value for the mass absorption coefficient of copper,[2] and this may be con-

[2] COMPTON, A. H. X-rays and electrons.

sidered the cutoff point. Substantially all of the radiation incident was included between these points, the greatest intensity lying within the $K_a - K_b$ doublet region for tungsten, between 0.21 and 0.18Å.

Several very curious effects were observed among the seedlings, which may be summarized here.

Premature flowering

Premature flowering is not infrequently observed in citrus seed beds, particularly in cases in which the acidity of the soil has not been properly

FIG. 2. Premature flowering of a chlorophyll deficient grapefruit seedling. Dosage 1300 r. in seed stage.

regulated. In the present case, however, the habit was pushed to an extreme which proved very interesting, especially in view of the fact that the hydrogen ion concentration of the seed beds was maintained within limits which, in the opinion of the citrus growers, should discourage early flowering.

Among one group of grapefruit seedlings arising from seeds which had been given x-ray dosages of from 300 to 1300 roentgens in both the wet and

dry states under the conditions described, two came into flower within six weeks of the time of sprouting. The flowers matured rather slowly, but, although diminutive, they proved to be normal in form. The plants, as shown, still carried the first pair of leaves intact, as is usual with citrus seedlings of this age. The plant shown in figure 1 was normal in leaf and flower coloration, and the leaves were nearly normal in form although somewhat elongate and diminutive. The other seedling (fig. 2) was extremely deficient in chlorophyll and the flower was imperfectly pigmented, being of a yellowish white color similar to the leaves. The stamens, however, were golden. The green plant received a dosage of 300 roentgens and the white one a dosage of 1300 under the same conditions.

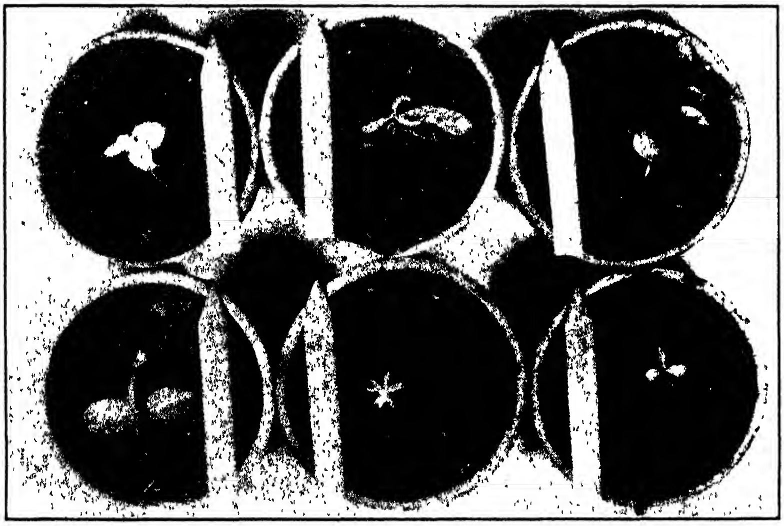

FIG. 3. Albinism of citrus seedlings following x-ray treatment in the seed stage.

The plant deficient in chlorophyll died very shortly, as was to be expected. The normally pigmented seedling, although deficient in roots, survived and grew slowly, and, when one year of age, again showed a flower bud on new growth, although it did not come to maturity.

At the age of nine months, 10 per cent. of this entire planting came into blossom almost simultaneously, the flowers in this case being of normal petal number and almost of normal size. Some of the flowering seedlings were of normal stature for their age, while others were distinctly dwarfed.

Albinism

Albinism, like premature flowering, is a character not infrequently apparent among citrus seedlings, especially in highly hybridized strains. In

the present case, however, an abnormally high percentage of seedlings showed this deficiency. It ranged from a condition best described as virescent, in which by no means all green pigment was lacking, through partial deficiencies in which a deep green was mottled irregularly throughout the leaf on a white background (fig. 3), to the complete whiteness characterizing one of the flowering types mentioned in the last section, and very many plants otherwise normal in appearance. Although albino plants were not absent from control plantings (as they very rarely are), the percentage of deficiencies in treated groups was manyfold higher.

Fasciation

Numerous cases of terminal and lateral bud fasciation were observed, the most pronounced of which is illustrated in figure 4, right. This speci-

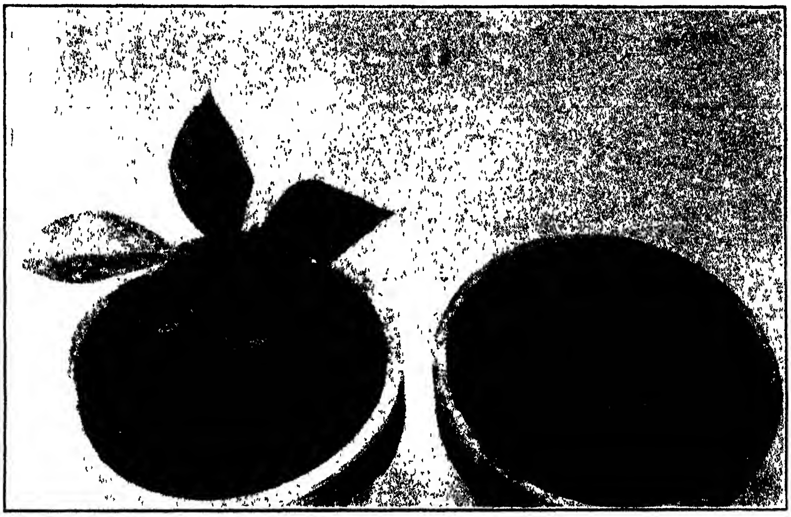

FIG. 4. Bud fasciation in *Citrus aurantium* following x-ray treatment of seeds. Control at left.

men of *Citrus aurantium,* when photographed, was but little over an inch in height. Control specimens, as is usual with this species, grew vigorously, and had attained a height of 10 inches when the photograph was taken. Clearly the primordia only of the later true leaves were affected, since the plant appeared normal in the first few weeks of life. Curiously the later leaves, although diminutive, were of nearly normal form.

Twisting

Two young seedlings from the time of sprouting showed a decided tendency to spiral in a counter-clockwise direction. Both plants twisted so markedly as to bend the stem from the vertical and to crush the leaves

against the stem (fig. 5, extreme right). After six months the habit was abandoned and later growth was normal. Both plants showed some evidence of tissue inversion and other characteristic x-ray injury during early

FIG. 5. Spiral twisting of stems of citrus seedlings following x-ray treatment of seeds. Control at left.

life. It is possible that the condition was brought about by x-ray induced abnormal mitoses.

Duplication

Very many instances were noted in which the midribs of leaves were split near the base, both halves being nearly equally robust. In a number

FIG. 6. Leaf modifications in citrus seedlings following x-ray treatment in the seed stage. See also fig. 5, center.

of cases, especially with the grapefruit, the entire leaflet was duplicated, the resulting two terminals usually being of nearly the same dimensions (fig. 5, center, and fig. 7). In lemon stock similar developments gave rise to partially bifoliate, bifoliate, partially trifoliate, and truly trifoliate leaves, often coexisting on the same plant (fig. 6).

FIG. 7. Grapefruit seedling with modified leaf, and accessory cup-like leaf. X-ray treatment given in seed stage.

Peloric leaf formation

An interesting development of a cup-shaped type of peloric leaf occurred in two grapefruit seedlings from x-rayed seeds, in each case the leaves proceeding laterally from the stem at a point some distance below the terminal bud. The formation was new in the experience of the writers and in that of a citrus horticulturist who examined it. The peloric leaves persisted for more than a year, the plants bearing them being otherwise normal.

Work is being continued in the field, and through the kindness of Mr. W. J. PLATTEN, of Sebastian, Florida, grafts of several of these variants have been made on to rough lemon stock, with a view to checking any possible modifications in fruit.

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CAROTENE AND XANTHOPHYLL IN PINEAPPLES[1]

O. C. Magistad

The color of the flesh of the fruit in pineapples varies from white or colorless to a deep yellow. The commercial varieties in the Territory of Hawaii, the Cayenne and Hilo, are both intermediate in shade. Within these varieties depth of color varies greatly from place to place, and even from fruit to fruit within a single field. The present investigation was undertaken to determine the nature of the pigments in the fruit of pineapples and to measure the quantity of pigment present.

Experimentation

Using the chemical methods of separation based on solubilities, as given by Willstätter and Stoll (10), Schertz (7, 8, 9), and Palmer (6), it was found that carotenes and xanthophyll were present in pineapple flesh together with anthocyanins, flavones, and traces of chlorophyll. The yellow colored shell of pineapple fruits was found to be relatively rich in carotenes and flavones, while the green leaves contained in addition to chlorophyll even larger concentrations of flavones, carotenes, and xanthophylls than were found in the fruit flesh.

Methods.—The finely chopped fresh material was extracted with acetone and the pigments present were transferred and concentrated in ether. Flavones were removed by washing with a 1 per cent. aqueous solution of sodium carbonate. Chlorophyll was saponified and separated from the carotenes and xanthophylls. The ether was evaporated off at temperatures below 40° C., and the carotenes and xanthophylls were separated in the usual manner on the basis of solubilities in methyl alcohol and petroleum ether.

Small violet red crystals formed on pouring a petroleum ether solution of carotene into methyl alcohol. Crystals also formed on evaporation of carbon bisulphide solutions of carotene. These appeared under the microscope to be identical with those described by Willstätter and Stoll (10, plate III). Carotene isolated from pineapple tissue gave a blue coloration with the antimony trichloride reagent of Carr and Price (2, 3).

The amount of chlorophyll present was estimated by comparison against a synthetic solution as advocated by Guthrie (4).

The quantity of carotene present was determined by colorimetric comparison with standard yellow Lovibond slides as described by Schertz (7). When compared with solutions of potassium bichromate as advocated by

[1] Published with the approval of the Director as Technical Paper no. 73 of the Pineapple Experiment Station, University of Hawaii. This paper was presented at the Boston meeting of the American Society of Plant Physiologists, December 28, 1933.

PALMER (6), fair agreement was obtained. A weighed quantity of pure carotene obtained from the Smaco Corporation when dissolved in petroleum ether gave readings checking well with the curves of SCHERTZ and of PALMER, and with the solution of carotene isolated from pineapples.

Xanthophylls were determined by colorimetric comparison against standard yellow Lovibond slides as advocated by SCHERTZ (8).

AMOUNTS OF PIGMENTS PRESENT

A medium sized pineapple plant with a ripe fruit was analyzed for the pigments chlorophyll, carotene, and xanthophyll. The results obtained are shown in table I.

TABLE I

PIGMENT CONTENT OF A PINEAPPLE PLANT

PLANT PART	FRESH WEIGHT	CHLOROPHYLL		CAROTENE		XANTHOPHYLL	
		PER 100 GM.	TOTAL	PER 100 GM.	TOTAL	PER 100 GM.	TOTAL
	gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Lower leaves	1056	65.50	691.68	3.75	39.60	2.05	21.65
Middle leaves	1598	50.65	809.39	1.85	29.56	1.43	22.85
Upper leaves	1012	48.00	485.76	2.38	24.09	0.71	7.19
Shoots ..	1162	36.25	421.23	2.38	27.66	1.17	13.60
Crown	145	26.58	38.54	1.05	1.52	1.20	1.74
Fruit stalk	151	3.95	5.96	0.28	0.42	0.27	0.41
Stem	1215	1.45	17.62	0.06	0.73	0.06	0.73
Flesh	1786	Trace	..	0.15	2.68	0.03	0.54
Core ..	214	"	....	0.20	0.43	0.04	0.08
Shell	930	"	....	0.33	3.07	0.33	3.07
Total	9269		2470.18		129.76		71.86

A study of this table shows first that the chlorophyll content of the relatively thick leaves of pineapple plants is lower than for thin leaves of common trees and plants. Thus, WILLSTÄTTER and STOLL (10, page 102) give values up to 300 mg. chlorophyll per 100 gm. green tissue for some plants. Again most plants contain more xanthophyll than carotene in their leaves, but in table I the pineapple plant is shown to have more carotene than xanthophyll throughout all its parts. The absolute quantity of carotene and xanthophyll present is small in comparison with most plants (10, page 102). Of special interest is the relatively low content of xanthophyll in the fruit flesh.

CAROTENOID PIGMENT CONTENT OF A NUMBER OF VARIETIES AND HYBRIDS OF PINEAPPLE FRUITS

A study was made to determine the quantity of carotene and xanthophyll present in the fruit flesh of various pineapple varieties. The fruits

were all grown in the Territory, those other than Cayenne and Hilo being grown by the Genetics Department at the substation at Wahiawa. The results are listed in table II.

TABLE II
CAROTENE AND XANTHOPHYLL CONTENTS OF FRUIT FLESH OF DIFFERENT PINEAPPLE VARIETIES

VARIETY	NUMBER OF FRUITS	PER 100 GM. FLESH		BOTH	RATIO C/X
		CAROTENE	XANTHOPHYLL		
		mg.	*mg.*		
Cayenne					
Lot 1	13			0.13 ± 0.004	
Lot 2	9	0.22 ± 0.011	0.026 ± 0.005	0.25 ± 0.012	8.5
Lot 3	2	0.15	0.011	0.16	13.6
Lot 4	12	0.18 ± 0.014	0.015 ± 0.002	0.20 ± 0.014	12.0
Hilo	24			0.15 ± 0.008	
7966	10			0.50 ± 0.03	
Natal	10			0.45 ± 0.03	
Ruby	10			0.41 ± 0.04	
Queen	5			0.38 ± 0.03	
7898	3			0.38	
8053	10			0.26 ± 0.02	
4552	11			0.17 ± 0.012	
8680	11			0.044 ± .0006	
Bermuda	10			0.031 ± 0.005	
8232	5			0.01	

The first part of the table gives the variation in pigment content of four lots of Cayenne. In these lots the carotene plus xanthophyll content varied from 0.13 to 0.25 mg. per 100 gm. of fruit flesh. Not infrequently fruits will contain even less than 0.13 mg. pigment per 100 gm. flesh. The data in the latter part of table II were obtained by estimating carotene and xanthophyll together, without separation.

A comparison of Cayenne with the other varieties indicates that some contain far more, and others much less pigment. Thus 7966, Natal, Ruby, Queen, and 7898 contain about twice as much pigment as does Smooth Cayenne; while some varieties and hybrids, such as 8680, Bermuda, and 8232 are almost devoid of pigment and appear very white-fleshed.

The range in carotenoid pigment content of pineapple fruits of various varieties is strikingly similar to the range observed by BILLS and MCDONALD in the carotene content of ten carrot varieties (1). They found Early Scarlet Horn to contain 9.6 mg. of carotene per 100 gm., while Isbell's Maude S on the other end of the series contained only 0.12 mg. carotene per 100 gm. Thus the most colorless carrot contains about as much pigment

as does Cayenne pineapple, while some carrot varieties contain about 40 to 60 times as much.

Permanence of carotene and xanthophyll in canned pineapple

During 1933, three lots of pineapple packed as indicated in table III were opened and their pigment content determined. The results appear in the table.

TABLE III

Carotene and xanthophyll content of stored canned pineapples

Lot	Number of samples	Per 100 gm. flesh		Ratio C/X
		Carotene	Xanthophyll	
		mg.	*mg.*	
1927 Pack, tins	10	0.088 ± 0.007	0.011 ± 0.001	8.4
1927 Pack, glass	10	0.121 ± 0.004	0.017 ± 0.002	7.1
1932 Pack, tins ...	18	0.097 ± 0.004	0.015 ± 0.006	6.5

No record is available of the pigment content of the pineapple fruits represented in table III at the time of canning. However, since the amounts present are about equal to the amount found in fresh pineapples, it can tentatively be concluded that there is no marked loss of carotene and xanthophyll during storage. The 1927 pack in tins and in glass was a homogeneous lot at the time of packing and should have had the same pigment content at that time. The greater quantity found in the glass packed material on examination in 1933 suggests that there is less decomposition of pigment when packed in glass as compared with tin.

Discussion

The yellow color of pineapple flesh is due to the presence of carotene and xanthophyll. These pigments are soluble in the fat solvents within the chromoplasts. Carotene predominates over xanthophyll. No attempt has been made to determine which carotenes were present nor the proportion of each.

A number of recent investigations have proved carotene to be a precursor of vitamin A. The β carotene is markedly superior to α and γ carotene. As a source of vitamin A, pineapples in this respect (**2, 5**) rank far behind such vegetables as lettuce, spinach, and carrots.

Summary

Pineapple fruits owe their yellow color to carotene and xanthophyll, carotene predominating. The quantity of carotene present ranges from about 0.10 to 0.25 mg. per 100 gm. flesh.

The writer wishes to express appreciation to Dr. M. B. LINFORD for aid in this study, and to Dr. J. L. COLLINS for samples of the hybrids.

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BRIEF PAPERS

LIGHT AS A FACTOR INFLUENCING THE DORMANCY OF LETTUCE SEEDS[1]

Lettuce seeds of the highest vitality may fail to germinate when tested in a closed chamber germinator. Seed analysts have observed that germination may be induced in many of these dormant lettuce seeds by presoaking them in cold water for 2 hours. However, such increase in germination is due not to the soaking in water but to the action of light on the wet seeds.[2]

Recent investigations by the writer show that it is even unnecessary to soak the seeds in cold water since the dormant condition can be broken merely by placing the seeds in an atmosphere saturated with water vapor and giving a longer exposure to light. Lettuce seeds of the Grand Rapids variety which were placed in a humid atmosphere and exposed to light for 10 hours and then placed on moist blotters at 20° C. in the dark germinated 98 per cent. A duplicate test which had received the same treatment but which was not exposed to light germinated only 8 per cent. The period of exposure to light can be shortened to 2 minutes or perhaps less by allowing the seeds to take up moisture from the air before exposing them to light. This germination response is of importance because it shows that water in the form of a film surrounding the seed is not essential to the breaking of the dormant condition by light.

Whether or not lettuce seeds in which the dormant condition has been broken by exposure to light will remain non-dormant when dried depends upon the physiological condition of the seed, the period of exposure to light, the temperature, and the manner in which the drying process is conducted. Lettuce seeds of the Big Boston variety which were placed in a humid atmosphere and exposed to light for a period of 24 hours at 23° C. and, without drying, placed on moist blotters at 20° C. in the dark, germinated 98 per cent. A similar lot of seeds which had received the same treatment but which was dried in the dark germinated 35 per cent. The failure of lettuce seeds to retain the non-dormant state when dried appears to be a characteristic response of lettuce seeds which require a temperature of 22° C. or lower for germination. It has been determined that if the seeds are set over water, exposed to light for 6 hours, and then placed in a refrigerator at a temperature of 6° C. for 10 days, they can then be dried and still retain the non-dormant state. Furthermore, it has been observed that

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 47.

[2] Shuck, A. L. Some factors influencing the germination of lettuce seed in seed laboratory practice. New York State Agr. Exp. Sta. Tech. Bull. no. 222. 1934.

exposure to light at the time of drying may have a marked influence on the percentage of dormant seeds. Moist Grand Rapids lettuce seeds which were exposed to light for 24 hours and then dried at room temperature in the dark germinated 92 per cent. at 20° C. A similar lot of seeds which were dried at the same temperature but in the light germinated only 5 per cent. The degree of dormancy originally existing in the seed appears to be the most important factor determining the necessary length of exposure to light and the germination response of the seed when dried.

The shorter wave lengths of the visible spectrum are more effective than daylight in producing a dormant condition in both presoaked and humidified lettuce seeds when they are again dried in the presence of light. Grand Rapids lettuce seeds were placed over water, exposed to light for 24 hours, and then dried in the light, in the dark, and under plates of spectral glass. The seeds were dried for 24 hours and then placed to germinate on moist blotters at 20° C. in the dark (table I).

TABLE I

INFLUENCE OF LIGHT AND DRYING ON THE RETENTION OF THE NON-DORMANT STATE IN GRAND RAPIDS LETTUCE SEEDS

TEST NO.	CHECKS, GERMINATION IN DARKNESS	PERCENTAGE OF SEEDS GERMINATING AT 20° C. IN DARK WHEN DRIED UNDER THE FOLLOWING LIGHT RELATIONS				
		IN DAYLIGHT	UNDER SPECTRAL GLASS			IN DARKNESS
			YELLOW	GREEN	BLUE	
	%	%	%	%	%	%
1	15	32	21	75	16	89
2	6	15	24	59	17	64
3	11	7	17	67	8	90
4	13	5	10	79	12	92
5	15	30	34	67	17	95
6	30	18	16	79	15	98

In one of the representative tests the seeds dried in the dark germinated 89 per cent., while those dried in the light germinated 32 per cent. Similar lots which were dried while exposed to the individual colors of the spectrum germinated as follows: violet 12 per cent., indigo 5 per cent., blue 16 per cent., green 75 per cent., yellow 21 per cent., orange 24 per cent., and red 15 per cent.

If the dormant state has been broken to such an extent that the seeds remain non-dormant when dried in the dark, then only a small percentage

of the seeds revert into dormancy when dried while exposed to the green rays, whereas if they are dried while exposed to either the red or the blue rays, a high percentage of the seeds become dormant. The violet, indigo, and blue rays are more effective in producing dormancy than the longer red rays since a semi-stable condition can frequently be produced in non-light-sensitive seeds by drying the moist seeds while exposed to the shorter wave lengths of light. The main factor influencing the germination response of seeds when dried while exposed to the different colors of the spectrum appears to be the degree to which dormancy has been broken, which state in turn is influenced by the temperature and the period of exposure to light. In order to duplicate these results it is suggested that the moist lettuce seeds be given an exposure to light for a period of 24 to 48 hours, since an exposure for only a few hours may not be sufficient to break the stable condition in the embryo while a prolonged storage over water will cause an increase of dormancy and finally deterioration of the seeds.

The influence of light on the germination of lettuce seeds has been studied by FLINT,[3] who has found that the longer wave lengths of the visible spectrum promote germination while the shorter wave lengths are not only ineffective in promoting germination but may inhibit it. Since the red rays are effective in breaking the dormant condition and promoting germination while the blue rays cause the formation of a semi-stable condition, it appears that there may be an intermediate region of the spectrum which has no appreciable influence on certain physiological processes in the embryo associated with the dormant state. That there is a region of the spectrum between the blue and the red rays which has less influence on the embryo than the rays on either side is suggested by the germination response of lettuce seeds after they are dried while exposed to the green rays. These observations have been made upon Grand Rapids lettuce seeds which usually exist in a dormant condition which makes them particularly sensitive to light. A corresponding semi-stable condition may or may not be present to such an extent in other varieties of lettuce seeds, and this fact must be taken into consideration when studying the response of different lots of seeds to light.

Lettuce seeds are continually changing organisms which show different degrees of dormancy depending on the age or physiological condition of the seeds. The seeds often exist in a dormant condition in which certain of the life processes necessary for germination appear to be in a state of delicate equilibrium. This condition can be broken by exposing the moist seeds to light. A similar unstable condition can be formed again by drying the seeds in daylight or by exposing them to the shorter wave lengths of the

[3] FLINT, L. H. Light in relation to dormancy and germination in lettuce seed. Science **80**: 38–40. 1934.

spectrum. These transformations can be made in the light and without the presence of water in the form of a film surrounding the seed. It appears that under these conditions light does not facilitate the passage of any substance from the seed but causes certain unknown photochemical changes within the seed.—A. L. SHUCK, *New York State Agricultural Experiment Station.*

A CASE OF CHANGE OF TROPIC RESPONSE

(WITH ONE FIGURE)

During a recent trip into the northern woods of Minnesota the peculiar formation seen in the accompanying illustration (fig. 1) was encountered. This specimen of the black spruce was found near the shore of Lake Hole-in-the-wall, a small lake which is located about six or seven miles southeast of Marcel. The two lateral branches had each made a three-quarter turn and then grew almost directly upward. The upper portions of these two branches if seen alone would appear much like the top of the main axis of the tree, with laterals extending outward and somewhat drooping.

Since the lateral branches of the black spruce normally grow nearly at right angles to the vertical main axis and perpendicular to the force of gravity, they may be said to exhibit plagiotropic response. No attempt, however, was made to determine the specific influence or influences responsible for this position. After making the turn the branches apparently became definitely negatively geotropic like the main trunk.

The writer is not certain as to the cause of this abnormal formation but thinks that it might have been caused by the mistletoe, *Arceuthobium pusillum,* which is known to attack the black spruce in Minnesota and produces abnormal development. At the time of the visit to this region he was unaware of the occurrence of this mistletoe and failed to look for its presence on this individual tree which was the only one seen which seemed to be abnormal. These two branches, however, are thickened at the curvature and resemble mistletoe injury as reported by colleagues who are familiar with its pathogenic symptoms.

Aside from the peculiar unique formation, is the fact that these branches are seemingly unaffected in their subsequent growth and have later responded to growth forces in such a manner as to resemble the main axis of the tree, illustrating quite definitely a change of tropic response. Just what forces were brought into play to cause the branches to grow in such a way as to form the circle it is difficult to say. The parasite must have stimulated growth on the upper and outer sides of the curve and then, after the growing tip had passed the half circle mark, left the elongating portion unaffected. After that, the end of the branch completed the turn until it

FIG. 1. Abnormal branches of the black spruce (*Picea mariana*) which have changed their direction of growth to resemble the vertical main trunk of the tree.

became upright and thereafter continued to grow vertically. One would think that the branch, once it had reached the half circle mark, would have continued growing horizontally, for at this point it seems no longer affected by the parasite. This horizontal position would have been its normal orientation. Why it responded to gravity or other forces in a manner different from that of normal branches after making the half turn is problematical, but it seems quite certain that there had occurred a change of tropic response after the elongating tip portion of the branch had passed the half turn resulting in the later vertical growth.—W. J. HIMMEL, *University of Nebraska.*

NOTES

Pittsburgh Meeting.—The eleventh annual meeting of the American Society of Plant Physiologists was held at Pittsburgh, December 27–29, 1934, in conjunction with the meetings of the A. A. A. S. The scientific programs were held in the Cathedral of Learning, University of Pittsburgh. There were four sessions for reading of papers, and joint meetings with the Horticulturists, Section G, A. A. A. S., and a joint symposium on plant hormones with the Physiological Section of the Botanical Society of America.

Business meetings were held by the Executive Committee on call by the President, and matters already in shape for action were attended to in the regular business meetings. The reports showed that the last year has been one of real progress. Membership is again increasing, and the finances are in sound condition.

The annual dinner was held at the University Club on December 27, with more than 100 in attendance. The dinner was made the occasion for election of corresponding members, announcement of awards, and a memorial address on Dr. KARL RITTER VON GOEBEL which was prepared by Prof. FRANCIS E. LLOYD, McGill University. In the enforced absence of the author of the memorial address, it was read by Dr. F. M. ANDREWS, Indiana University. This address will be published in PLANT PHYSIOLOGY in a later issue. At the close of the festivities, President LIVINGSTON was tendered a memorial of affection and appreciation by graduates of Johns Hopkins University in honor of his completion of 25 years of service to plant physiology at Johns Hopkins. Those who were present will treasure the memory of these unforgettable events.

Summer Meeting.—A summer meeting at Minneapolis and St. Paul late in June is in prospect. The arrangements will be made by the Minnesota Section, and members will be informed more fully in the April number of PLANT PHYSIOLOGY as to the exact time of the meeting, hotel accommodations, extent of the program, and social events if any. The summer meeting of the A. A. A. S. is scheduled for Minneapolis, and this circumstance provides an opportunity for a meeting with our oldest Section. Previous experience has demonstrated that our Minnesota members and friends are past masters in the arts of entertainment and organization of meetings. In extending a cordial invitation to all of the members of the Society to come to Minnesota, they hope that visitors may come not only from the middle west, but also from more distant regions.

Corresponding Members.—The second election of corresponding members of the American Society of Plant Physiologists occurred at Pittsburgh.

Two distinguished plant physiologists were honored: Dr. GOTTLIEB HABERLANDT of the University of Berlin, and Dr. V. N. LUBIMENKO of the Botanical Garden of the Russian Academy of Sciences at Leningrad. Both of these men have enjoyed long and active careers in plant physiology, and have made notable contributions to the advancement of our knowledge of plants and their behavior. The Society has honored itself in honoring them.

Life Membership.—The tenth award of the CHARLES REID BARNES life membership in the Society was made to Professor FREDERICK FROST BLACKMAN, Fellow of St. Johns College, University of Cambridge, and for many years Reader in Botany at Cambridge. He has been a Fellow of the Royal Society of London for nearly thirty years, and has been the guiding spirit of the physiological work at Cambridge for several decades. This is the first time that the award has been made to a scientist residing outside of North America. No happier choice could have been made at this time.

Stephen Hales Award.—The fourth award of the STEPHEN HALES prize was made to Dr. CHARLES A. SHULL of the University of Chicago. This prize was established at the Nashville meeting in 1927, and the previous prizemen are Dr. D. R. HOAGLAND, University of California; Dr. W. W. GARNER, U. S. Department of Agriculture; and Dr. H. B. VICKERY, Connecticut Agricultural Experiment Station. These men constituted the committee on the award for 1934. In the absence of the chairman of the committee, the citation was read by Dr. CHARLES F. HOTTES, University of Illinois, and the certificate of award was presented by the President, Dr. BURTON E. LIVINGSTON.

Program Committee.—The program committee for the St. Louis meeting in December, 1935, has been appointed. The personnel of the committee as announced by President LIVINGSTON is as follows: Dr. E. S. REYNOLDS, Missouri Botanic Garden, chairman; Dr. H. R. KRAYBILL, Purdue University; Dr. S. V. EATON, University of Chicago. The secretary-treasurer, Dr. A. E. MURNEEK, University of Missouri, is *ex officio* member of the committee.

Editorial Board.—At the end of our first decade, the constitution and by-laws of the Society are being carefully examined by the executive committee for possible improvements. Many changes are likely to be proposed. One of the most important changes will affect the size and constitution of the editorial committee for the official journal, PLANT PHYSIOLOGY. Provision will be made for appointment of some of the members of the editorial staff by the executive committee, and for election of some members by the

Society. The editorial staff will be enlarged possibly to eight members, five appointive and three elective for definite terms, so that more fields, such as horticulture, forestry, etc., can be represented on the board. Constitutional changes will wait on approval of the changes by ballot of the members; but by-law changes are put into effect on unanimous approval by the executive committee.

Cost of Reprints.—Arrangements are being made for a reduction in the price of reprints. In order to effect any saving to our members it will be necessary to have full cooperation of authors of papers. The prompt execution of orders at the time galley proofs are returned to the editorial office is a part of the plan. Blanks giving the prices charged according to length of the reprint will be attached to the galleys, and blanks are included for ordering reprints and for shipping directions. As in the past, 50 free reprints without covers will be added to the orders; but in case the author orders no reprints no gratis reprints are to be sent. Authors should keep these provisions in mind in making up orders.

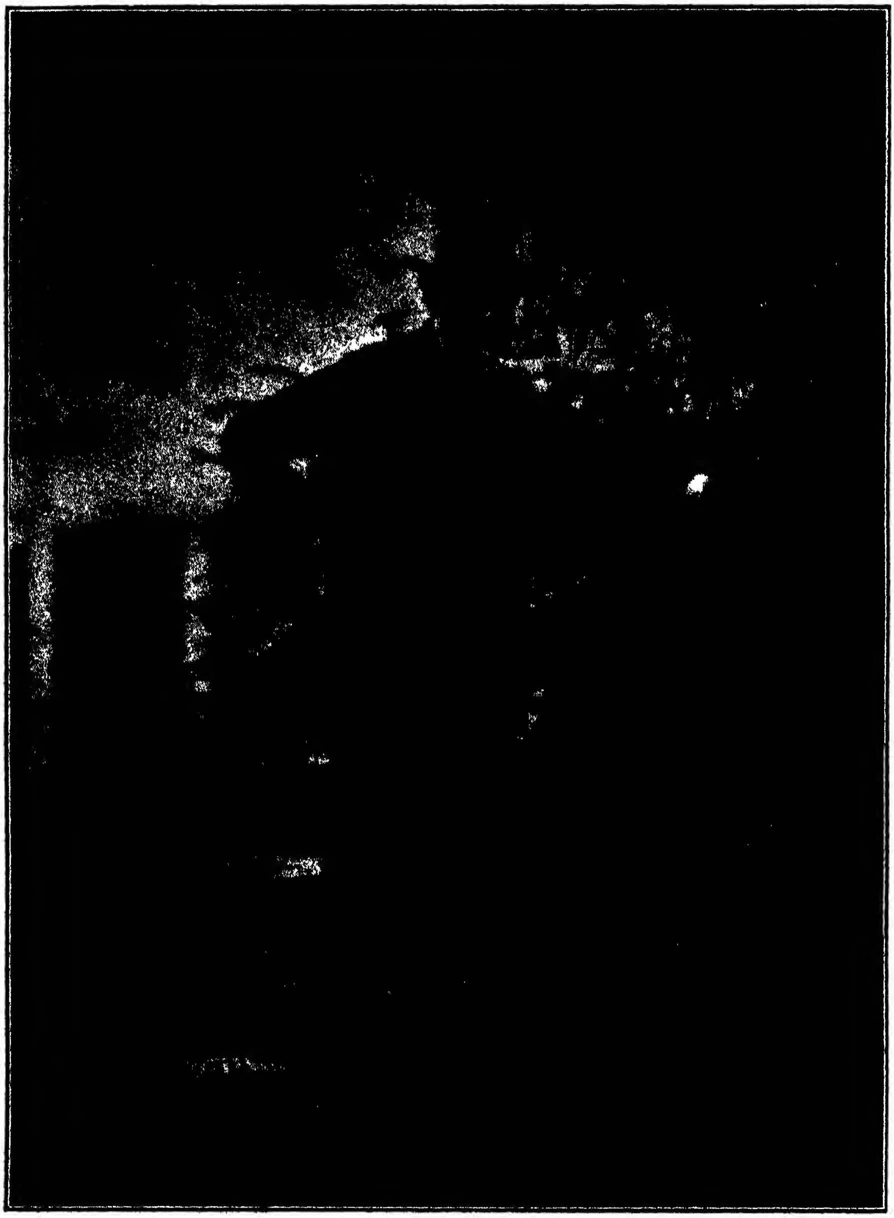
Endowments.—Three of the endowment funds of the American Society of Plant Physiologists are in excellent condition. The general endowment is very much too small. The Society is in position to accept and administer gifts and bequests in accordance with the wishes of the donor upon the approval of acceptance of gifts for special purposes by the executive committee. An approved form for bequests will be sent to any one interested in making bequests to the Society. Cash gifts to the general endowment are welcome at any time, and will be put to work at once upon receipt. Occasional gifts have been made to the endowment during the last several years, and it is hoped that more friends may assist in the development of the Society in this practical way.

Portraits.—With the appearance of the April number of PLANT PHYSIOLOGY, two additional portraits will be added to the collection of prints available for distribution. The full set of 20 reprints of portraits may be had for $2.00 plus postage and insurance.

Water Relations Reprints Wanted.—A new edition of "The plant in relation to water" by N. A. MAXIMOW is in preparation. The author of this work would appreciate it very much if he might receive reprints of important contributions on this subject from all American investigators who have worked in this field. The international character of scientific knowledge makes such cooperation very desirable. Those who wish to assist him should address Professor N. A. MAXIMOW, Institute of Grain Farming, Postal Agency 20, Saratov, U.S.S.R.

Statistical Methods.—The fifth edition of R. A. FISHER's Statistical Methods for Research Workers has been published by Oliver and Boyd, Tweeddale Court, Edinburgh, or 33 Paternoster Row, London E.C. The changes are again insignificant. Section 5 is now a historical note; section 49.1 on analysis of covariance has been enlarged; section 21.01 considers YATES's correction for continuity; and 21.02 gives the exact treatment of 2×2 tables. It is a useful work for those who are obliged to master and use statistical methods in research. Most investigators would find it advisable to be familiar with the practical usefulness of statistics in testing their results. The price of the volume is the same as for the earlier editions, 15 shillings net.

Excretion and Secretion.—Volume 32 of the Monographien aus der Gesamtgebiet der Physiologie der Pflanzen und der Tiere is entitled Die Stoffausscheidung der höheren Pflanzen. The author is Dr. A. FREY-WYSSLING of the Federal Technical Highschool, Zürich. The introduction sets forth the scope of the work, and defines the various forms of elimination of materials from the plant body. Section I considers the deposition of cell walls, the substances laid down in them, and the structures that appear, such as the micellae, intermicellar spaces, and intermicellar deposits; the physiology of the cell walls is summarized in the latter part of this section. Section II deals with "recretion," the removal of inorganic elements that have been taken in from the soil, function for a time in the plant, and are then deposited in crystalline or other insoluble form. Calcium salts and silicon dioxide deposits are the two main "recretions." Section III deals with excretion proper, and section IV with secretion. A brief summary closes the text. There is a bibliography of about ten pages, a subject index, and 128 figures. It is a useful summary of the last stages of metabolism in plants. The book may be obtained from the publisher, Julius Springer, Berlin, at RM 28 for brochure copies, and RM 29.4 for cloth bound.



KARL RITTER VON GOEBEL
1855–1932

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KARL RITTER VON GOEBEL

FRANCIS E. LLOYD

(WITH PLATES I AND II)

It is now just two years since KARL VON GOEBEL passed into the beyond, and it is peculiarly fitting that this Society—The American Society of Plant Physiologists—should select him for especial remembrance at this time.[1] While avowedly a plant morphologist, he was in very deed as much a physiologist directing his efforts at the elucidation of plant form. But more than a botanist, he was a man of extraordinary personality. We honor him today for his influence on our science and thought.

Born in 1855 in Billingheim, Baden, his early youth was overshadowed by illness and the circumstances following the death of his father in 1860. Following his destiny fixed upon by a pious mother, he was educated for the Ministry, but when he was doing his duty as a theological student at Tübingen, he took advantage of the presence of HOFMEISTER to pursue the study of Botany. His predilection grew out of his earlier love of nature awakened in him by his environment, especially the Schwabian Alp, which "won all his love" and first aroused in him an appreciation of nature. It is easy to imagine the influence which HOFMEISTER had on the young GOEBEL, but it led to the grave difficulty of disappointment for his mother; and it was only after three years that the die was cast. In 1876, HOFMEISTER becoming ill, GOEBEL went highly recommended to DEBARY in Strassburg and a year later was promoted to the doctorate. Some months were then spent at the Zoological Station at Naples, after which followed his military obligation in Wuertzburg, whither he was drawn by the presence there of SACHS. What a sequence of scientific fathers: HOFMEISTER, DEBARY, SACHS! Developmental morphology, anatomy, physiology under the masters! And their seed fell on good soil. GOEBEL may be said with much truth to have come in the fulness of time—the great

[1] Annual Meeting, Pittsburgh, December, 1934.

foundation of the science had been laid and it was his privilege to build on that foundation. So well did he work that he became assured of rapid success. In 1881 he became First Assistant at Leipzig, in the same year he was called to Strassburg as Professor Extraordinarius, then to the like status at Rostock with the understanding that he should very soon be made Ordinarius. "Here," GOEBEL writes, "I found nothing but an herbarium; no garden, no institute. Salary 2,400 M. The appropriation available 250 M. a year. My 82-year-old predecessor remarked to me, 'My dear GOEBELSCHEN, I have always sent this amount to my daughter. I suppose it will do little harm if we continue to do so?' It cost me much effort to bring into being a modest institute and a small garden, but I learnt much from the experience."

In the winter of 1885–6 after getting these matters started, FRITZ OLTMANNS was called to be GOEBEL's assistant and *locum tenens* during his first foreign journey to India, Ceylon, and Java. Next year he was called to both Leipzig and Marburg. Choosing Marburg and liking it well, he planned to end his days there. There he undertook the editorship of *Flora,* which furnished a daily stint of work for the rest of his long life, for the task passed from his hands only with his death. A second journey, this time to South America, yielded much adventure and his descriptions of the Venezuelan paramos in the *Schilderungen.*

But his dream of living out his life in Marburg was not to be. In 1891 he went to München where botany had been for a time in a state of lassitude. And it was here that, beginning with his thirty-sixth year, he carried out his great life-work. It was here that we outside of his Fatherland knew him best. Some of us can recall the meager and poorly equipped Institute near the center of the city within a stone's throw of the *Hauptbahnhof*: GIESENHAGEN in a little room off the main laboratory crowded with students, GOEBEL in his own laboratory, not much larger than GIESENHAGEN's, and a little lean-to glasshouse. A small but richly planted garden furnished an apparently endless supply of material. GOEBEL's plans for an adequate glasshouse had been frustrated by a congeries of circumstances but with the happier final result that Nymphenburg was chosen by GOEBEL as the site for the Institute and Garden and, with the support of his friend the King, these were brought to fruition and constitute a material memorial to him and his life-work. The Garden was designed to serve not only science but the people. Every day during the growing season sees troops of school children wandering here and there, orderly and under guidance one may be sure, satisfying their rampant curiosity for which there is endless material, the *Biologische Abteilung* and the glasshouses being the chief attractions. For the public in general, an extensive formal garden with a lily pool gives delight. To GOEBEL this Garden was his realm, and to his students it was and is the source of a

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wealth of material of biological significance. The extensive range of glass-houses have collections which in their relative importance and arrangement bear the stamp of the master's thought. He was particularly proud of the treefern house—and a beautiful thing it is,—but scarcely less of the tropical and larger central house. I have the impression that a photograph here reproduced (plate I) taken of him standing on the rough steps of the central rock-mass in this house among his beloved plants, was the one he liked best; but this is only an impression. Plate II shows him seated in his library.

The new Institute was not altogether to his liking. Always impatient of showiness, the "*Prunk*" of the entrance hall frankly bored him. A place for work—this was his object, and here from 1913 on he worked with the same relentless striving that marked his earlier period.

But the material monument is of less importance than that of the mind. Over 200 publications record his observations and thought. This merely quantitative statement is, however, inadequate. Most of his publications were fairly lengthy, a number of them lengthy, and some massive. The *Organography* alone has the stature of an encyclopedia, but without its perfunctory character. It is a mine of information illuminated by a brilliant light of thought. So all-embracing are the contents that, wherever it may end, all future morphology must take account of, if not begin with, the *Organography*.

All this was the product of an almost fanatically restless mind, restless above all in the acquisition of facts from the study of living plants. It was hunger for materials which took him on long and trying journeys into the tropics, into New Zealand, Australia and South America, whence he brought back extensive collections of material. Of his thorough work in the field I am personally aware, since he turned over to me in most generous fashion the whole of his *Utricularia* collections which contained materials in fluid of no less than about forty species. Keen as was his interest in this genus, it was scarcely less so in many others which he must have collected with equal zest. His last lengthy journey, to Java and Sumatra, was undertaken when approaching 70 years of age and while on shipboard he received a shower of congratulatory telegrams. Shortly after, a *Festschrift* containing contributions of his students and friends did much, I imagine, to heal the wounds of the sad period of the world war.

Though made Emeritus in 1931, he had to carry on for yet another year at the age of 77 when his successor, Professor Fritz von Wettstein, took over. It was characteristic of him that his penultimate lecture ended the course; he thus avoided the demonstration which would inevitably have occurred. Even after retirement, as had been his habit through the years past, he made each day a tour of the glasshouses, did his editorial work and carried on investigation, and was beginning, even at that age, a study

of the placenta, which started when he cut in two a *Catalpa* fruit with his penknife and asked me what the huge placenta meant biologically.

To speak briefly of GOEBEL's work is difficult and a general characterization must here suffice. As everyone knows, it covered an extraordinarily wide range of observation on an array of materials which was made available only by his own explorations in most parts of the world. He thus became aware of the exceeding variety of plant form. But he was not content merely to observe in the restricted sense; he felt the urge to explain form in physiological terms, how it comes about and to what end. To this purpose he was constantly experimenting, to be sure in the simplest sort of way: "For an experiment one needs a plant, a flower-pot with earth and a question," the "question" implying brains!

Though he was little given to speculation, his wide and intimate knowledge of plant form led him to a modification of the Darwinian selection theory. He was convinced that the variety of plant form was much greater than the variety of the conditions under which they grow, and saw in these various products many structures which could not be regarded as directly adaptive, but rather indifferent, being neither harmful nor useful. They can arise or disappear without being subject to selection, or they can group themselves and combine to produce members which may enable the plant to become adapted to quite other conditions than the primary ones, and the principle here implied was one of his chief guides in reflecting on the form relations of the plant.

It is also to his merit that be broke away entirely from the formal morphology of which GOETHE was the chief exponent. It was this freedom from prejudice which enabled him, *e.g.*, to lead us to an understanding of that most curious and puzzling group, the Lentibulariaceae, particularly *Utricularia,* acute observation and close reasoning being exemplified in rare manner in his discussion of the evidence available. It was a mark of his reasoning that he insisted on the distinction of objectivities and names. "First of all let it be understood that in nature there are neither 'leaves' nor 'lateral members.' Both ideas are but abstractions of our minds, not just the expression of the facts of observation." Unless we see this as truth, "morphology stiffens into a dead schematism," that is, "if we do not regard the plant to be what it is in reality, a living body whose functions are carried out in the closest possible relations to the outside world." Thus morphology became a physiology: "Morphology is that which we do not yet understand physiologically."

As indicated, his methods of work were of the simplest, and he could be pretty caustic when, for example, one used a microtome when a hand-section would do the work as well or better. I heard him remark in the earlier days of microtome sectioning that anyone who could cut microtome sections regarded himself as a botanist. To be sure,

there was a microtome in his laboratory: "Das ist ganz schön, aber auch dieses muss mit Sinn angewandt werden; eine blosse Hobeltechnik genügt nicht zu morphologischen Schlüssen."

He always insisted on an adequate historic treatment of a subject and he was thoroughly impatient of work done in ignorance of what had gone before. His waste basket was the richer. But he was still more impatient of inaccurate observation. One might differ from him in a matter of explanation, but if one fouled his anchor chain of observation his criticism was a flaming sword.

"Dazu sei nochmals bemerkt, dass es sich nicht um eine *Theorie,* sondern um *Tatsachen* handelt, für deren Richtigkeit ich gerne auch weiterhin die Verantwortung übernehme und von denen sich jeder überzeugen kann, der eine etwas schwierige entwicklungsgeschichtliche Untersuchung auszuführen versteht."

But let it not be thought that he was an overbearing man. On the contrary, he had a placid manner and was kindness itself in his daily relations. His personality was of a rare kind; he walked the earth with a god-like serenity, and indeed his stature, massive head and level eye started a tradition among the natives of Java with whom he came in contact, so that later travellers heard that a god had been there before them. His beard may have been a factor in establishing the tradition, but yet we confess to a sympathetic appreciation of the feeling that in GOEBEL we saw something *übermenschlich.*

While he walked and talked with kings of the earth, he remained quite unspoiled. When his King made him a knight and his students next morning hailed him with an unusual amount of academic applause, he remarked with his peculiar dry humor, that although he had been made a knight he would still come to his lectures afoot. Of course, it could not be otherwise, for his was a simple if extremely intelligent mind. Honors recorded elsewhere were poured in on him but, while appreciating them, he was not puffed up.

Generous, he gave of his time and material to whomsoever he found worthy. If he was found in error he was readily willing to admit it without abatement of his kindness. A brilliant lecturer, given to a rich dry humor, he depended none the less on the things which he discussed, and the students' desks were always loaded with fresh materials for observation while he spoke of them. His lecture room often looked like a horticultural show.

The favorable season during the summer semester permitted excursions and these usually to the nearby Tyrol, when he was the most genial companion. Even then he had an ailing heart, so that he climbed slowly; otherwise his long legs had set a hard pace for some of us. I recall on a beautiful sunny day, on the swelling bosom of one of the high hills sur-

rounding Innsbruck, finding *Botrychium lunaria,* I think it was, searching with him for an hour for the prothallia without success but with much enlightening talk in which BRUCHMANN was not forgotten. Such excursions, usually for two days, spending the night at a country inn, were a full expérience for a young botanist of the western world. GOEBEL was the center of interest about which the international group of students played: it was always "*der Geheimrat.*"

And it was while on an excursion to his beloved Schwäbische Alb, whither he ever returned, that he fell and received a severe injury from which, had his heart not failed him, he would have recovered. He died on October 9th, 1932, at the ripe age of seventy-eight years, after being only a few months out of harness but still active as President of the Bavarian Academy of Sciences. I cannot but think that his latter years were made happier for him when, during the last International Botanical Congress at Cambridge in the company of Professor L. JOST, after visiting STEPHEN HALE's rooms in Trinity, we visited the Chapel and there looked in silence at the memorial to the German students of Cambridge who had died fighting for their country.

His only visit to North America was on the occasion of the International Congress of Arts and Sciences, September, 1904, held under the able guidance of Professor HUGH MÜNSTERBURG, and it was then that we had contact with GOEBEL on our own soil. That occasion was made memorable by his presentaton of his paper "The present-day problems of plant morphology," from which brief quotations have been made above. It should be realized that this was a sort of confession of faith, and for its comprehensive values it should be read by every aspiring botanist no matter in which direction his inclinations lie. It is a masterpiece of clear reasoning. At such a gathering of notable scientific men from all parts of the world, memorabilia inevitably emerged; it is appropriate to mention one to this audience in particular. It was the occasion of an informal dinner fittingly enough held in the "Tyrolean Village," even if a synthetic one, at which were seated VON SCHRENCK, JOHN M. COULTER, HUGO DEVRIES, F. O. BOWER, CHARLES B. DAVENPORT, W. G. FARLOW, WILLIAM TRELEASE, and our own guardian spirit, CHARLES R. BARNES. The modest GOEBEL was the dominant figure there.

May his memory as a man live in our hearts and his teachings in our minds. *Forsan et haec olim meminisse juvabit.*

Note:—In preparing the above memorial I have had the benefit of the notices by Professor FRITZ VON WETTSTEIN, Prof. F. O. BOWER and Professor G. KARSTEN.

MCGILL UNIVERSITY
MONTREAL, CANADA

PROOF OF THE PRINCIPLE OF SUMMATION OF CELL E.M.F.'S

H. F. ROSENE

(WITH FIVE FIGURES)

The distribution of electric polarity in the unstimulated, uninjured root was first demonstrated by LUND and KENYON (**6**) in the roots of *Allium cepa, Eichhornia crassipes,* and *Narcissus.* Observations of the electric polarity of a number of other roots have been made by the writer and will be presented in detail in a separate paper. A distinctive feature of the electric polarity common to all the roots so far examined is the occurrence of a characteristic distribution of E.M.F. per unit length of root.

During the measurements of E.M.F. in the roots of different plants, it was observed that when a drop of water was placed around a region of the root between and not at the electrode contacts, the magnitude of total E.M.F. was altered. The water appeared to act as a shunt, changing the IR drop of the length of root over which the potential difference was being measured. This observation, and the fact that water is a necessary part of the environment in which roots can grow, led to the present study which was undertaken to determine the magnitude and direction of change in electric polarity produced by "liquid shunts" around the root.

Since more is known about the electric phenomena of the onion root (*A. cepa*) than about any other, it was selected as the experimental material. Observations made by LUND and KENYON (**6**), MARSH (**8**), and the writer on hundreds of different roots show that the electric polarity of the onion root changes from time to time. The root may manifest a stable potential difference for hours and then a steady increase or decrease in E.M.F. may appear, or it may exhibit rhythmic fluctuations of E.M.F. It has sometimes been observed that if, at a certain time of day, the roots of one bulb from a group of onions which were set at the same time exhibit rhythmic changes in electric polarity, the roots of most of the bulbs in this set will also manifest rhythm; at another time the roots of all the bulbs will exhibit a stable potential difference. From these facts it is obvious that in any analysis of the bioelectric potentials of the root tip the following must be taken into consideration: (*a*) the form of the curve of distribution of E.M.F. per unit length of root tip at a particular instant; (*b*) the variation of the characteristic form of the curve of distribution from instant to instant; (*c*) slow drifts of increase or decrease in potential difference between any two points which may occur; (*d*) occasional rhythmic fluctuations in E.M.F. which may vary in duration and magnitude from time to time. The changes mentioned in (*b*), (*c*), and (*d*) occur when all known external

conditions are maintained constant. They are therefore conditioned by changes within the root itself. Hence in any experiment in which E.M.F. is modified by external conditions, it is necessary to distinguish between the spontaneous changes which are determined by causes of internal origin and the changes produced by altering the external conditions.

In order to determine whether the observed change in total E.M.F. of the root tip produced by surrounding a given region between the electrode contacts with tap water is uniquely characteristic of the system of maintained bioelectric potentials which is a distinguishing feature of electrically polar tissues, experiments were also made on the injury potential of nerve. It is a familiar fact that a potential difference between the cut end and the longitudinal surface of a nerve or muscle may be established and maintained for some time under suitable experimental conditions. It is also a familiar fact that the injury potential varies with the concentration of ions at the electrode contacts; but the writer knows of no work which shows that this E.M.F. may be modified by placing a conducting medium around an uninjured region between and not at the contacts, as will be shown to be the fact in the root.

Apparatus and method

The apparatus adapted for use in this investigation has been described in a previous paper (**13**). To surround a given region of the root with liquid, a glass cup (diagram M in figure 1) was used, which consisted of a piece of glass tubing, 5 mm. in outside diameter, with a small cover slip cemented on one end to form the bottom of the cup and a capillary tube cemented to a hole at one side at the base, to serve as a delivery tube. The cup was admitted into the moist electrode chamber through the opening *M*, shown in figure 2 of ROSENE and LUND's paper (**13**). By means of a micromanipulator, the shunt cup (as it will be called) could be raised into position around the root which penetrated through a small hole in the cover glass that formed the bottom of the cup, or the holder with the bulb could be lowered by a different micromanipulator and the root passed through the cup. The liquid was admitted into and withdrawn from the shunt cup through the capillary tube which was connected to a reservoir. The water flow was controlled by the reservoir stopcock and by raising or lowering the rack and pinion stand supporting the reservoir. The apparatus permitted delicate control of all the manipulations and environmental conditions of the root.

The roots were grown in tap water. Those which were selected varied from 50 to 80 mm. in length. All of the roots but one were removed from the onion bulb just before it was placed in the electrode chamber. The experiments were run at room temperature and measurements of electric potential were made by a Compton electrometer.

The general procedure was as follows: The root was placed in the electrode chamber and contacts were made by using the micromanipulators to move the glass claw projections of the electrode cups into position around the root (see fig. 4, 2A and 2B, ROSENE and LUND **13**). The preparation was allowed to rest for a short period and the distribution of potential was then determined by moving the positive electrode (to quadrants) at the tip toward the negative electrode (grounded) near the base, in steps of 1 or 2 mm., making a reading at each step. The positive electrode was then moved away from the root while the empty shunt cup was placed in position (as in diagram M, fig. 1). A horizontal microscope with ocular micrometer was used to determine the position of the contacts on the root and to make the measurements of length. With the shunt cup in position, observations of the electric behavior of the root under constant external conditions were made for a short period and then liquid (tap water or paraffin oil) was run into the shunt cup from the reservoir. The height of the liquid in the cup was determined by the horizontal microscope. With careful manipulation, no leakage of liquid from the cup occurred and mechanical stimulation was reduced to an insignificant minimum. The length of the period during which the liquid was retained in the cup surrounding the root was varied in different experiments, and sometimes in a single experiment. Throughout each experiment readings were made at 15-second or 1-minute intervals.

Experiments and results

PROCEDURE 1

ALTERNATE ADDITION AND REMOVAL OF WATER FROM SHUNT CUP

Figure 1, B, is the curve of distribution of potential along a root tip which does not exhibit a negative potential in the region 5–10 mm. from the tip. The diagram of the root below curve B is drawn to the same scale as the abscissa and shows the exact position of the shunt cup, *S.C.*, also drawn to scale, as well as the position of the electrodes which are indicated by arrows. In this experiment a shunt cup 2 mm. in height was employed. It surrounded that region of the root which exhibits the highest electropositivity. Curve A shows that each time the segment of the root surrounded by the cup was filled with water, an immediate and abrupt drop in E.M.F. occurred; and each time the water was withdrawn the E.M.F. of the root was abruptly increased. The magnitude of increase of E.M.F. did not quite equal the magnitude of decrease. This was evidently due to a film of water which remained on the root and acted as a partial shunt when the water was withdrawn from the cup.

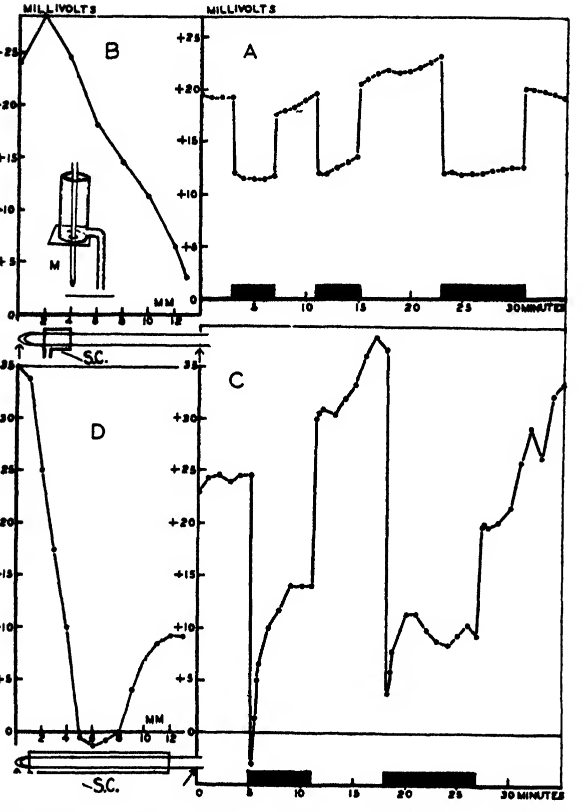

FIG. 1. Effect of liquid shunt around a segment of the root between the electrode contacts on the observed E.M.F. of the root. B and D are the curves of distribution of E.M.F. of two different roots obtained by moving in 2 mm. steps, the positive electrode from the tip toward the negative electrode which was stationary at 14 mm. from the tip. Abscissa gives the position of the positive electrode in mm. from the tip. The diagram below each curve, drawn to the same scale, shows the exact position of the shunt cup (*S.C.*) and electrode contacts (designated by arrows) during each experiment. Curves A and C show changes in E.M.F. when tap water is alternately added to and removed from the shunt cups in B and D respectively. Heavy portions on base lines A and C indicate duration of period when cup is full of water; intervals between when it is empty. Inset M, below curve B, shows shunt cup in position around the root. See text for description.

The height of the larger cup, which was used in the next experiment, was 11 mm. Its exact position on the root is shown in the diagram below curve D, which gives the distribution of potential of that root. The shunt cup (*S.C.*) covered most of the region between the electrode contacts. As indicated by curve C, as soon as water rose upward from the bottom of the shunt cup, there was a sharp decrease in E.M.F. and the electric polarity

of the root was suddenly inverted. During the period when the jacket was filled with water, the E.M.F. increased and normal orientation of the electric polarity was restored; but the potential difference was maintained at a low level. Removal of water produced a sharp rise in potential which reached a new high level. The potential difference continued to increase during the period that the cup was empty. Repeating the procedure produced a similar change in the magnitude of E.M.F., but the electric polarity of the root was not inverted. Similar slow fluctuations of E.M.F. occurred when the shunt cup was filled with water and when it was empty. The abrupt changes in E.M.F. produced by the addition and removal of water from the jacket are readily distinguished from the rhythmic fluctuations of E.M.F., which are associated with changes in the internal processes of the root, by the fact that the abrupt changes in the first case produce an immediate rise in the curve and in the latter case a definite slope is evident. A comparison of curves A and C shows that a greater magnitude of change in E.M.F. was produced in the second experiment. This is explained by the difference in the length of the "liquid shunt" in the two instances, which corresponds to the difference in the height of the water column of the different cups, and also in part by the fact that the total E.M.F. of the root region (14 mm. long) between the electrode contacts was greater in C.

Procedure 2

Addition and removal of water from the shunt cup in steps at definite intervals

When the distribution of E.M.F. was a single unidirectional gradient of fall of potential along the root, as indicated by figure 2, curve B, and water was added to the shunt cup at definite intervals, thus increasing the height of the water column around the root in steps, the E.M.F. of the root was correspondingly altered in steps. This fact is illustrated by curve A, figure 2. Each arrow which points downward indicates that the height of the water column in the cup was raised 1.5 mm., and each arrow which points upward indicates that the height of the water column was lowered 1.5 mm. The positions of electrode contacts and the shunt cup on the root are illustrated by the root diagram B′ below the curves of distribution, figure 2. As shown by curve A, the E.M.F. was diminished each time the water level in the cup was raised by adding more water and it was increased each time the water level of the cup was lowered by withdrawing water. In other words, as the length of the liquid shunt was increased or decreased, the E.M.F. was correspondingly increased and decreased. After the cup had been filled with water, the observed electric polarity of the root re-

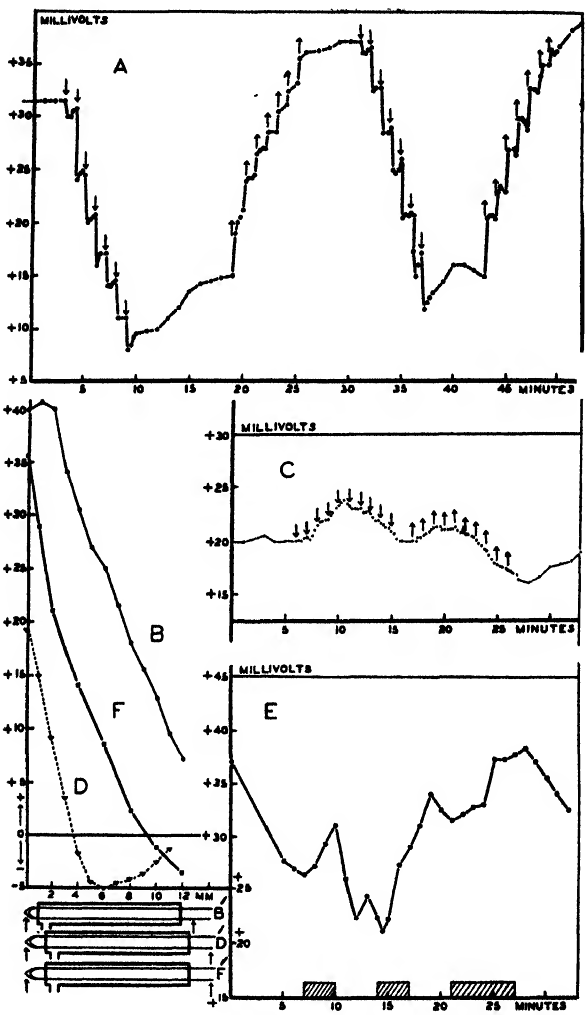

FIG. 2. (*a*) Effect on E.M.F. of root when the liquid shunt is increased and decreased by increments, at successive intervals, and (*b*) absence of effect when paraffin oil is added to and removed from shunt cup. The curves are from three different experiments on three different roots. Arrows pointing downward in curve A show when height of water column in shunt cup was increased by 1.5 mm. increments; arrows pointing upward, when it was decreased in a corresponding manner. Arrows pointing downward in curve C show when height of oil column in shunt cup was increased in steps of 1 mm., arrows pointing upward, when it was correspondingly decreased. Shaded intervals on base line in E indicate when shunt cup was filled with paraffin oil; intervals between, when it was empty. B, D, F are curves of distribution of electric potential for each root and correspond to A, C, and E respectively. Diagrams of the roots B′, D′, F′, below the curves of distribution, show exact respective positions of shunt cup and electrode contacts (arrows) in each experiment.

mained at a relatively lower level; and after the cup had been drained it remained at a higher level. The curves which are represented by figures 1, C, and 2, A, were obtained under conditions in which the height of the water column in the full cup was the same in each case, and the length of the region of the root over which the E.M.F. was measured was also the same.

PROCEDURE 3

ADDITION AND REMOVAL OF PARAFFIN OIL FROM THE SHUNT CUP

In order to determine whether the observed change in E.M.F. of the root produced by adding tap water to the shunt cup could also be produced by adding a non-conducting liquid, paraffin oil was substituted for water.[1] Figure 2, C, shows the results obtained when procedure 2 was followed. The arrows pointing downward indicate the time at which the level of paraffin oil in the cup (which was empty at the beginning of the experiment) was raised in steps of 1 mm. The arrows pointing upward indicate the times at which the oil was removed in a corresponding manner. The curve illustrates the fact that the root exhibited rhythmic fluctuations in E.M.F. but did not show the striking change in E.M.F. which was produced when water was added and removed from the cup around the root under similar conditions, as shown by curve A, figure 2. The corresponding curve of distribution of electric potential for this root is given by figure 2, D, and the position of the jacket by diagram D′, below the curve.

Figure 2, E, was obtained when the shunt cup was at alternate intervals filled and drained with paraffin oil. Throughout the experiment, the root manifested a distinct rhythm in electric behavior, but it shows no quick change as a result of adding and withdrawing oil from the cup. This is clearly evident when the curve is compared to curve C in figure 1, which was obtained by a similar experimental procedure using water instead of oil. The perpendicular changes in curve C, figure 1, which express the alteration of E.M.F. when water was added to or removed from the cup, are entirely absent in curve E, figure 2, when tap water is replaced by the non-conducting oil.

PROCEDURE 4

ADDITION AND REMOVAL OF WATER FROM THE SHUNT CUP WHEN POSITION OF CUP AROUND THE ROOT IS CHANGED

Figure 3, B, shows the distribution of potential over 25 mm. of a root tip which exhibits two regions with opposed electric polarities. Figure 3, A, shows the curve obtained when the cup was placed, in turn, in two dif-

[1] The paraffin oil was non-toxic, since, when the roots were replaced in water, they grew in a normal manner.

ferent positions corresponding to the regions of opposed polarities and was alternately filled and drained at intervals while in each position. The first part (below the bracket (a) of curve A, figure 3) was obtained when the cup was near the apical contact, as illustrated in the upper root diagram B′ below the curves of distribution, and the second part of the curve (below the bracket b) was obtained when the jacket was in a position near the basal

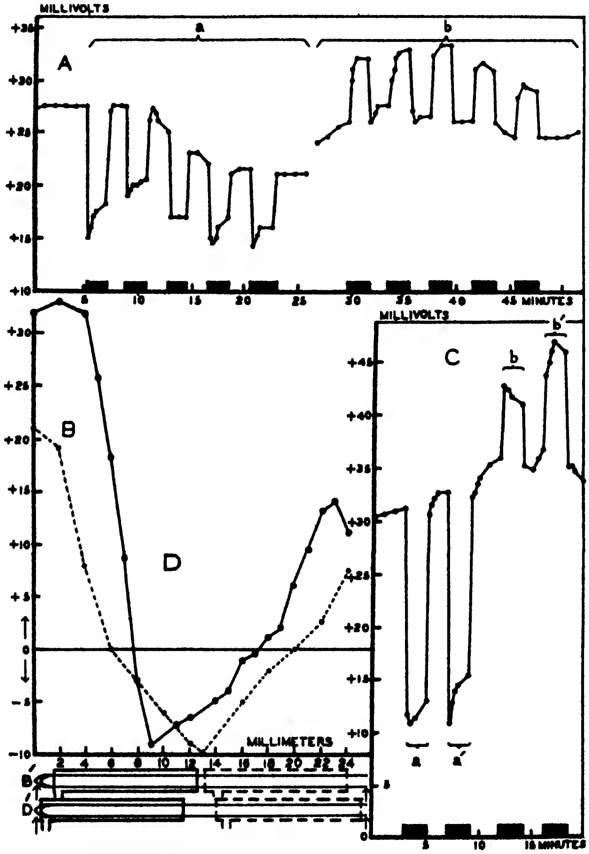

FIG. 3. Effect of placing a liquid shunt on different regions of opposed electric polarities. Curves B and D show distribution of potential in two different roots. Upper root (diagram B′) below the distribution curves shows exact positions of electrode contacts (arrows) and two successive positions of shunt cup in experiments from which curves (a) and (b) in A were obtained. Lower root (diagram D′) is corresponding diagram for curves in C. In A and C the portions of the curves indicated by the brackets (a, a') were obtained when the shunt cup was in the position indicated by the continuous heavy outline of the cup on each root diagram; those portions indicated by brackets (b, b') when it was in the position indicated by the interrupted outline of the cup. Heavy portions of the base line of A and C show when the cup was filled with water; intervals between, when it was empty.

contact, as indicated by the dotted outline on the same root diagram. In each position the shunt cup was alternately filled and drained at 2-minute intervals. That portion of the curve under bracket (*a*) shows a *decrease* of the total E.M.F. of the root each time water was added to the cup, and an increase when it was removed. On the other hand, that portion of the curve under bracket (*b*) shows an *increase* in E.M.F. each time water was added to the cup and a decrease when it was removed. *The opposite effects on the electric polarity of the root with the cup in the positions corresponding to the regions of opposed polarities, show that the direction of change of E.M.F. produced by adding a liquid shunt is determined by the orientation of the electric polarity in the cells of the shunted region and also demonstrates that the two systems of opposed E.M.F.'s summate in an algebraic manner.*

In another root, following a similar procedure, the shunt cup was placed first at the apical contact and second at the basal contact, so that when it was filled, the entire length of root covered by the cup and the specific electrode contact near it were surrounded by water. In each case therefore a relatively extensive region at the electrode contact was shunted. The results are shown by figure 3, C; the curve of distribution of E.M.F. per unit length of root is shown by figure 3, D; and the positions of the cup and electrode contacts are indicated in the lower diagram of the root D′ below the distribution curves. In each position the cup was filled and drained at 2-minute intervals. As shown by the brackets (*a*, *a*′ and *b*, *b*′) in curve C, opposite changes in the electric polarity are observed in apical and basal ends of the root. The greater magnitude of the change shown by the positions of the curves (*a*, *a*′) occurred in the region of the root which exhibited the higher difference in potential.

In a third root, which also manifested the characteristic distribution of E.M.F. with a "valley" of negativity at 8–30 mm. from the apex, as indicated in figure 4, B, the cup was at successive intervals placed in three different positions on the root. It was then filled and drained once while in each position. The successive positions of the cup on the root are indicated below the curve of distribution of E.M.F. by diagrams a, a′, and b. Those portions of curve A, figure 4, which are indicated by the brackets (*a*, *a*′, and *b*), show the corresponding changes in electric polarity of the root when the cup was filled in each of the three positions. The greatest change in E.M.F. occurred when the region with the highest potential was surrounded by water. As in the preceding two experiments, the direction of change of E.M.F. was determined by the orientation of the electric polarity in the region upon which the shunt was applied.

These experiments indicate that if observations are made of the E.M.F. of a given length of root which manifests oppositely oriented polarity poten-

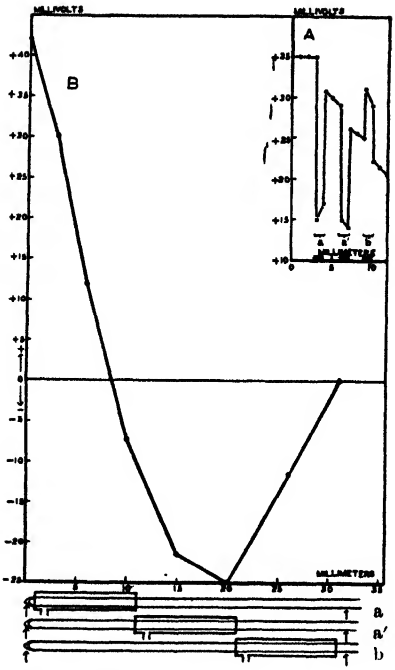

FIG. 4. Effect on electric potential, of addition and removal of a liquid shunt at three different positions on the root with a curve of distribution of E.M.F. as shown in B. Diagrams a, a′, and b below base line show successive and exact positions of shunt cup on same root. Arrows indicate positions of electrode contacts. The parts of the curve above the brackets (*a*) and (*a′*) and (*b*) in A indicate the change in E.M.F. produced by adding and removing water from the shunt cup in the positions corresponding to a, a′, and b of the root diagrams below.

tials, during an interval when the shunt cup filled with water is moved up or down the root, increase or decrease in E.M.F. would appear when the cup passed over a "hill" or "valley" of potential difference. In this way the orientation as well as the relative magnitude of E.M.F.'s per unit length could be determined.

Many such determinations on different roots were made, and the orientation and relative magnitude of E.M.F.'s per unit length was ascertained in this manner. In each case results were checked by comparison with the curve of distribution of E.M.F. per unit length obtained by moving the positive electrode toward the negative electrode as described above. When the empty cup or the cup filled with paraffin oil was similarly moved, no changes in E.M.F. were observed.

These experiments show further that we are dealing with a system or systems of E.M.F.'s, some of which at least have their origin in polar cells arranged in series. This point will be clear if the reader will refer to the

discussion and diagrams of cells in series and in parallel in LUND's paper (4) on the theory of cell correlation. In diagram I of figure 2, pages 285, LUND gives a simple hypothetical system of four cells, A, B, C, and D, arranged in series in which A is from the region of highest positive potential, B from a region just proximal to A, C from an isoelectric region, and D from a region of oppositely oriented polarity. When connected in series as illustrated, the total E.M.F. of the system would be equal to the algebraic sum of the E.M.F.'s of the cells between the contacts as represented by circuit 4 in the diagram (fig. 2, I, LUND 4). If, in such a system, a decrease is produced in the IR drop of cells A or B or both, the total E.M.F. of the system would necessarily fall; and if such a decrease is sufficiently great in magnitude the polarity of the system would be reversed since it would then be determined by the polarity of cell D. This is the explanation for the drop in potential shown in curves A and C, figure 3, and curve A in figure 4 when the conducting medium was placed around a region of high positive potential. If, on the other hand, a decrease is produced in the IR drop of cell D, the E.M.F. of which is opposing the P.D.'s of cells A and B, an increase in the total E.M.F. of the system will appear, as shown by the rise in potential in curves A and C, figure 3, and curve A, figure 4, which occurred when the conducting medium was placed around a region that exhibited a negative polarity potential.

PROCEDURE 5

SHUNT CUP OUTSIDE OF THE ELECTRODE JACKET

Very little or no change in E.M.F. of the root is observed when water is added to or removed from the shunt cup when placed outside of the electrode circuit. Figure 5, X and Y, shows the positions of the electrode contacts in two such experiments. The shunt cup in one experiment was filled with water during the first half of the experiment and with paraffin oil during the latter half. At 1-minute intervals the cup was alternately raised and lowered, thus surrounding 1.5 mm. of the extreme tip with water when raised. The position of the cup when over the tip is indicated by the interrupted outline in figure 5, X. The results are represented by curve A. The stippled portions of the curve show the intervals during which the apex dipped into the water-filled cup and the portions indicated by diagonal lines give the intervals during which the apex dipped into the oil-filled cup. Each time the water-filled cup was raised over the root, a small but definite drop in E.M.F. occurred, and each time it was lowered a small increase in E.M.F. was observed. No such change in E.M.F. was noticed when the oil-filled cup was similarly raised and lowered. In both experiments the effect was very small.

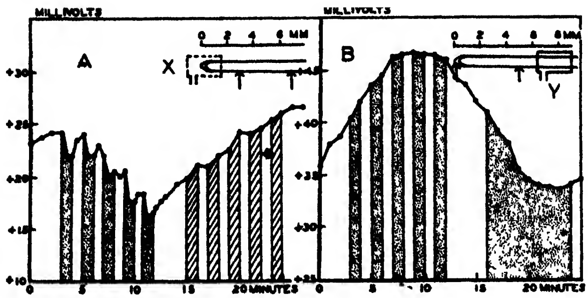

FIG. 5. Effect of tap-water shunt on E.M.F. of a given region when it is applied to a region of the root outside of the electrode circuit. Insets X and Y are diagrams of two different roots which show exact position of the segments of the root covered by the shunt cup, as indicated by the interrupted outline in X and the solid outline of the cup in Y. Stippled areas on the base line of A and B show when the shunt cup is filled with water, diagonal portions when it is filled with paraffin oil, and intervals between when the cup is empty.

In the other experiment the cup was placed in position around a region of the root relatively basal, as illustrated by figure 5, Y. It was filled with water during the intervals represented by the stippled portions of curve B, figure 5. Curve B indicates that no change in E.M.F. is produced by adding and removing water from the cup under these conditions.

Both experiments showed that the electric polarity of the root was in a fluctuating state manifesting rhythm. The small change in E.M.F. observed when the water-filled cup was raised and lowered indicates that part of the electric field at the apical electrode contact was included in the region covered by the cup when it was raised.

Experiments on frog nerve

The frog's sciatic nerve was carefully isolated and a thread tied around the proximal end by means of which the nerve was gently threaded through the openings of (*a*) the glass projection (contact) of the electrode connection to the quadrants, (*b*) the bottom of the empty shunt cup, (*c*) the glass projection (contact) of the grounded electrode cup, and finally fastened in a glass clamp which thus suspended the nerve in the moist electrode chamber. The electrode cups and glass projections were filled with frog Ringer solution. When both electrode contacts surrounded the uninjured surface of the nerve no potential was exhibited, but when the nerve was cut at the electrode contact (to quadrant) on the distal portion below the cup, an injury E.M.F. appeared. This E.M.F. was not subject to change by adding and removing Ringer solution around the region surrounded by the cup, nor did any effect appear when paraffin oil replaced the Ringer solution. Although the procedures described above in the experiments on the onion

root were repeated with different nerves, the presence of a liquid conductor around the uninjured surface of the nerve between the electrode contacts failed to produce an effect on the injury potential in any way comparable to that which was observed on the maintained E.M.F. of the polar cells in the root tissue. Evidently the systems of E.M.F. are fundamentally different in one or more respects; in the root tissue there is a system of cellular E.M.F.'s arranged in series (and probably in parallel also) such that the addition of a liquid conductor around cells between and not at the contacts will alter the total E.M.F. of that system, whereas in the injured nerve the system is closed and the addition of a liquid conductor between the contacts does not alter the E.M.F. In the latter case, the origins of the P.D.'s are probably limited to the electrode contacts. In the root electrically polar tissue is present, the cells of which are the seats of individual E.M.F.'s that summate algebraically to give the E.M.F. of that system.

Conclusions

The preceding experiments show that when tap water is placed around a region of the onion root between the electrode contacts the E.M.F. of the root is altered, but when paraffin oil is similarly placed around a region of the root no change in E.M.F. is observed. This fact indicates that when a liquid which contains electrolytes surrounds a region of the root the output of electric energy by the root is altered. The effect on individual cell E.M.F.'s is summated and expressed in the change of the electric polarity of the whole. A liquid shunt around the region of the root which exhibits a positively oriented unidirectional polarity diminishes the electric polarity of the whole, while a liquid shunt around the region which exhibits an oppositely oriented unidirectional polarity increases the electric polarity of the whole. This fact furnishes conclusive evidence that the total observed E.M.F. of the onion root is the algebraic sum of the definitely oriented E.M.F.'s of individual cells.

The fact that the E.M.F. between the cut end and the longitudinal surface of the frog sciatic nerve was not altered by following the procedure used in the experiments on the root shows that the observed effect produced by adding a liquid shunt to the root is uniquely characteristic of the system of continuously maintained bioelectric potentials, distinctive of the root cells.

The fact that the potential difference of the root may be increased or decreased by a liquid shunt indicates that the electric circuit system of the root is not a closed system. In its natural environment the root is exposed to soil or other solutions which contain electrolytes. Bioelectric currents flow outward into the surrounding medium from regions of high electric positivity in the root and currents flow inward from the surroundings to

regions which exhibit relatively low positivity or negativity. Accordingly, the observed phenomena are obviously significant in relation to the problems of (1) transport of ions, (2) absorption of water and solutes by the root, (3) transpiration, and (4) growth.

Energy changes are involved in the process of absorption, and many investigators have shown that permeability and osmotic relations alone are inadequate to explain the phenomena of absorption in the root (**1, 3, 9, 14**). The electric energy produced by the oxidative metabolism of the cells (**5, 13**) is continuously available for work and may be utilized by the root in the processes of absorption and transport. It has been shown that absorption of water and ions by the root is directly correlated with oxidation (**10, 2, 7**), and it has been demonstrated for the first time that the electric potentials of the root and oxygen tension are quantitatively interdependent (**6, 13**). A consideration of the fact that the electric circuit of the root is not a closed system, and the above mentioned relations (*i.e.*, (*a*) of bioelectric currents to oxidation and (*b*) of absorption to oxidation) indicates that these are linked phenomena.

The fact that electric energy is available for the transport of ions, and the additional fact that the E.M.F. of the root is modified only when an electrolytic solution comes in contact with the root, indicate that the available output of electric energy by the root is related to the conductivity of the solutions in which the roots grow. Root growth is dependent upon ion transport and upon the absorption of water and solutes. It may possibly differ in solutions of low and high electric conductivity.

A detailed discussion of the experimental results in relation to the phenomena mentioned above will be omitted, since the purpose of this report is to show that the magnitude and orientation of cellular E.M.F.'s is modified by an electrolytic solution around the root and that the electric circuit of the root is not a closed system, but attention is called to the fact that the study of electric behavior of the root establishes a precise and intelligible approach to the problems of (1) transport of ions, (2) absorption of water and solutes, (3) transpiration, and (4) growth.

Summary

1. The electric polarity of a given region of the root tip (*Allium cepa*) is decreased or increased when an electrolytic solution such as tap water (liquid shunt) surrounds a segment of that region.

2. The magnitude of change in electric polarity is directly related to the length of the liquid shunt, and the direction of change is determined by the orientation of the polarity potential in the segment to which the shunt is applied.

3. The level of E.M.F. manifested before the liquid shunt is applied is reestablished when the shunt is removed.

4. The observed changes in E.M.F. produced by the addition of a liquid shunt to the root are distinguished from the rhythmic fluctuations in E.M.F., produced by causes of internal origin, by the abrupt change in E.M.F. which occurs when the shunt is added.

5. The observed changes in E.M.F. are determined by the presence of ions in the applied solution. No change in E.M.F. of a given region of the root is observed when a non-conducting liquid is applied to a segment at that region.

6. The results indicate that the system of continuously maintained E.M.F.'s present in the root involves cells arranged in series so that their polar axes coincide.

7. When the liquid shunt is applied to a region outside of the electrode circuit no change in E.M.F. is produced.

8. There is an absence of effect on the injury E.M.F. of frog sciatic nerve following the same procedure used in the experiments on the root. The observed change in E.M.F. of the root, produced by the addition of a liquid shunt, is uniquely characteristic of the system of maintained cellular E.M.F.'s present in the root.

9. The observations furnish direct evidence that the principle of algebraic summation of E.M.F.'s in polar cell systems applies to the electric polarity of the onion root.

The writer wishes to express gratitude to Prof. E. J. Lund for valuable suggestions and criticisms. The investigation was aided by a grant from the research fund of the Department of Zoology, University of Texas.

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EFFECTS OF EXFLORATION ON PLANT METABOLISM

STANLEY AUSTIN

(WITH TEN FIGURES)

Introduction

The practical importance of the proper balance between the vegetative and reproductive processes in fruit crops has stimulated many horticultural investigations seeking to improve our practical control of these functions. Owing to the slow growth, large stature, and perennial habit of fruit trees as well as their delayed response to experimental manipulation, it has been difficult to secure comprehensive data directly. Consequently considerable work has been done on short-lived annuals, like tomatoes, the development of which is known to be comparable to that of fruit trees.

Use of herbaceous plants in horticultural studies has incidentally provided a considerable amount of agronomic data, and hence it has been natural for the agronomist to extend such investigations in his own field to important herbaceous crops not previously investigated. The existing extensive literature implies that an inverse quantitative relation exists between the reproductive and vegetative processes, growth usually tending to diminish as the size of the fruit crop increases (**4, 5, 7, 13, 14, 21, 22, 23**). It should be noted, however, that structural features of the plant in part determine the relationship between vegetative and reproductive processes. In plants of the determinate habit, the inflorescence utilizes all the apical meristem and hence arrests elongation. According to many investigators, the indeterminate habit, on the other hand, presumably permits stem elongation to continue until the developing fruits begin to monopolize the food supply. The fact that fruiting obviously interferes with elongation in crop plants of the indeterminate growth habit has led to the inference that all plants of this type behave similarly (**26**). It is difficult, however, to see how this interpretation would apply to indeterminate plants which normally set only a small quantity of fruit. Moreover, the rosette vegetative phase of certain long day plants such as spinach suggests that the photoperiod may be as important as reproduction in suppression of stem growth.

In short day plants exhibiting arrested elongation under short day conditions, it is not apparent whether the response is due to fruiting or only to the shorter day length. Although the statement has been made (**2**) that "the reproductive phase of growth apparently curtails the vegetative phase" in the soy bean, a preliminary investigation by the writer dis-

closed that exfloration of the soy bean, an indeterminate short day plant, did not result in increased growth. The present investigation was consequently undertaken to discover why exflorated soy bean plants did not behave like more typical species such as the tomato, which continue vegetative development indefinitely if exflorated.

Methods

Initial exfloration experiments with soy bean plants grown in the greenhouse seemed to indicate that the vegetative development was not influenced by continued removal of flowers. To determine whether this lack of response to exfloration might have been due to unfavorable conditions in the greenhouse, the experiment was repeated during successive seasons on plants grown out-of-doors. The results being entirely confirmatory, a more intensive investigation was undertaken. Data presented here are for two crops of plants, one grown in the summer of 1931 and another in the summer of 1932. Ito San soy beans were grown out-of-doors in well inoculated soil and all flowers were removed from one series of plants but left to develop on the control series. All plants were watered during periods of dry weather. Material was harvested for chemical analyses at frequent intervals, 20-gm. samples being preserved in alcohol for carbohydrate determinations and larger samples dried in an oven at 100° C. for dry weight, total nitrogen, and ash determinations.

Total organic nitrogen not modified to include nitrates was determined on samples of the dried material. The material preserved in alcohol was extracted several times with 80 per cent. alcohol (**37**), reducing sugars being determined on aliquots of the clarified extract and expressed as dextrose. Aliquots of the same extract were hydrolyzed with hydrochloric acid for the determination of total sugars, which are also expressed as dextrose. The residue from the alcoholic extraction was hydrolyzed by boiling for 2.5 hours with dilute hydrochloric acid under a reflux condenser. Reducing sugars were then determined and expressed as polysaccharides (**20**).

Samples of the dried material were ashed, dissolved in hydrochloric acid, and made up to volume for mineral analyses. Calcium was precipitated as oxalate and titrated with potassium permanganate. Phosphorus was determined colorimetrically by a modification of the Bell-Doisy colorimetric method (**3**). Magnesium was precipitated from the filtrate of the calcium determinations as magnesium ammonium phosphate and the phosphate content of the precipitate determined by the same method as used for phosphorus. Potassium was precipitated as potassium cobaltinitrite and weighed directly in Gooch crucibles.

Material for the 1931 crop was harvested when flowering began and a month later when the fruits had reached almost their full size. The samples designated as "leaves" represent composite samples of all the leaves on the plants. The stems were divided equally into upper and lower halves and are referred to as "upper stems" and "lower stems." Harvesting of the 1932 crop was begun when the plants were still very small and the data are more comprehensive, but unfortunately the plants were badly damaged by winds before the last harvest and their weights are not available. The "tips of stems" represent 2 to 3 inches of the tips of the stems.

FIG. 1. Similarity in appearance of mature exflorated and fruiting soy bean plant.

Data and discussion

Growth of normal Ito San soy bean plants proceeded rapidly for some time after flowering began, the check in the growth rate seeming to coincide with the enlargement of the fruits. The stem tips of rapidly growing plants were large and blunt, but as the plants reached maturity these tips became more and more slender and the new internodes became shorter until they finally ceased to grow.

Continuous removal of flowers, however, did not increase either height or diameter of the stems or size of the leaves. Growth stopped at the same time in the exflorated and the control plants. There was a slight wrinkling of the leaves of some of the exflorated plants. The similarity in appearance of the exflorated and control plants was striking (fig. 1). The ana-

TABLE I

Analyses of 1931 Ito San soy beans; percentages in terms of dry weight

	July 3, 1931			August 7, 1931						
	Blooming			Explorated			Controls			
	Leaves	Upper stem	Lower stem	Leaves	Upper stem	Lower stem	Leaves	Upper stem	Lower stem	Fruits
	%	%	%	%	%	%	%	%	%	%
Percentage dry weight	22.20	12.15	15.09	30.80	27.71	29.03	30.90	24.77	27.15	27.05
Total nitrogen	4.36	3.00	1.49	4.04	1.60	1.50	3.95	1.52	1.20	3.63
Reducing sugars	1.34	2.58	2.04	1.23	2.79	1.97	1.02	4.37	4.37	2.10
Total sugars	4.34	5.90	5.53	3.25	4.90	3.73	2.82	6.58	6.21	4.92
Polysaccharides	17.43	15.81	16.44	17.65	22.42	26.44	12.49	16.18	18.18	21.92
Total carbohydrates	21.77	21.71	21.97	20.90	27.32	30.17	15.31	22.76	24.39	26.84
Calcium	1.43	1.28	1.11	1.72	1.14	0.92	1.95	0.93	0.79	0.82
Magnesium	0.26	0.28	0.28	0.26	0.27	0.34	0.28	0.27	0.34	0.27
Phosphorus	0.43	0.36	0.35	0.29	0.27	0.27	0.28	0.21	0.28	0.43
Potassium	2.53	4.39	3.62	1.57	1.78	1.50	1.35	1.87	1.28	2.32

lytical data for 1931 are given in percentages of dry weight (table I) and in grams per plant (table II), and are illustrated graphically (figs. 2–5).

TABLE II

SOY BEANS, ABSOLUTE WEIGHTS OF CONSTITUENTS IN GRAMS PER PLANT

	JULY 3	AUGUST 7		
	BLOOMING	EXFLORATED	CONTROLS (STEMS AND LEAVES)	CONTROLS (PLUS FRUITS)
	gm.	*gm.*	*gm.*	*gm.*
Fresh weight	134.00	269.00	257.00	364.00
Dry weight	24.00	79.50	73.00	101.80
Nitrogen	0.795	2.23	1.94	3.03
Reducing sugars	0.439	1.44	1.97	2.54
Total sugars	1.21	3.01	3.36	4.79
Polysaccharides	4.03	16.73	10.83	17.15
Total carbohydrates	5.24	19.74	14.19	21.94
Calcium	0.314	1.10	1.03	1.23
Magnesium	0.065	0.222	0.212	0.295
Phosphorus	0.096	0.222	0.190	0.326
Potassium	0.785	1.28	1.07	1.78
C/N ratio	6.58	8.85	7.32	7.24
$\frac{\text{Moisture}}{\text{Potassium}}$	140.20	148.00	172.00	147.40

It has been shown by MURNEEK (**23**, **25**) that fruit production limits the growth of tomato plants and that defruiting or exfloration greatly increases the size of the plants so treated. Experimental exfloration has no such stimulating effect on the growth of the soy bean. This is a photoperiodic plant in which short day length apparently not only induces flowering but inhibits stem elongation as well. However, a shorter day seems to be necessary to affect the growth rate than to initiate flower formation, because growth continues for some time after flowering begins, long enough in fact to treble the dry weight of the stems and leaves, observations which are also confirmed by the work of other investigators. GARNER and ALLARD (**9**) found that Biloxi soy beans in a ten-hour day stopped growing as soon as flowering began, but under the influence of a 13-hour day they tended toward an everbearing habit so that growth and fruiting proceeded simultaneously. OWEN (**30**) observed that sterile soy bean plants became but little larger than those which set fruits, and that microchemical tests indicated the presence of an abundance of starch in the stems of these sterile plants.

NIGHTINGALE (**27**) believes that in *Salvia* assimilation of nitrogen is limited by a short photoperiod, and as a result carbohydrates accumulate in

the plants which then become fruitful because of the increase in the C/N ratio. Accumulation of carbohydrates in the stems of exflorated soy bean plants (figs. 6, 7) cannot be explained in this way because the soy bean is not limited in its ability to absorb nitrogen under short day conditions. This is evident from the data which show that about 36 per cent. more nitrogen is assimilated by the controls than by the exflorated plants under short day conditions during which fruiting normally occurs. Movement of

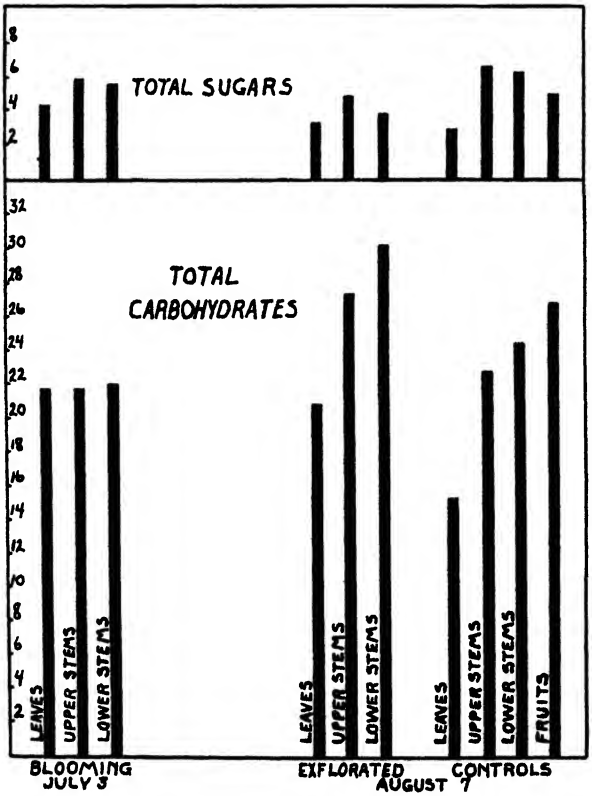

FIG. 2. Composition of soy beans; percentages in terms of dry weight.

nitrogen from the stems and leaves does not account for all of the nitrogen in the fruits. The analytical data not only show that the vegetative reserve is maintained but that more is taken in to supply the demands of the developing fruits. This interpretation agrees with the observation made by WEBSTER (**39**) that nitrogen in the soy bean is not massed in one part of the plant at the expense of any other part. Further confirmation of this condition is found in the data of BORST and THATCHER (**2**), which show that the absolute amount of nitrogen increases faster in the seeds than it decreases in the stems. GINSBURG and SHIVE (**12**) have also found that the

nitrogen content of normal soy beans was not appreciably altered by increasing the nitrogen concentration of the culture solution.

Data given in this report also indicate that nitrogen was not accumulated in exflorated plants, being but little higher than in the vegetative parts of control plants. On the other hand, the percentages of nitrogen were almost as high in the stems and leaves of exflorated and control plants as in flowering plants which were still vegetatively active. If lack of nitrogen assimilation had been the limiting factor in the growth of exflorated

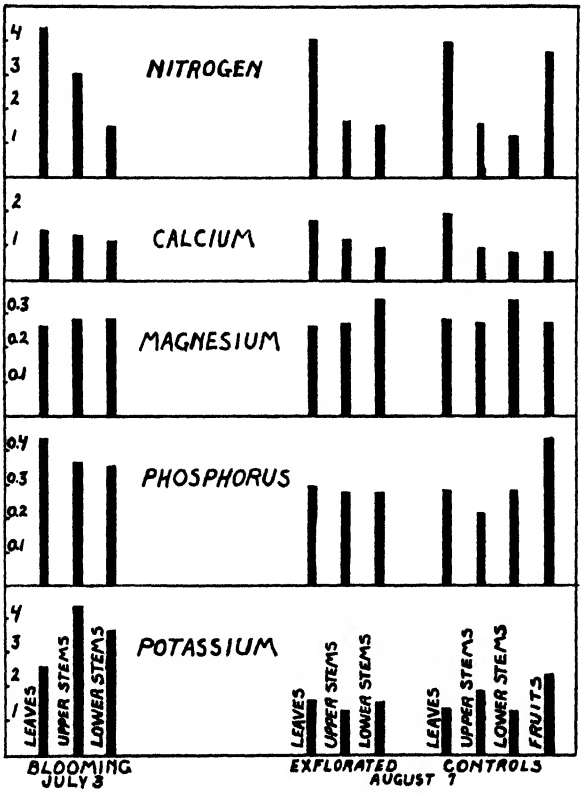

FIG. 3. Composition of soy beans; percentages in terms of dry weight.

plants, their nitrogen content should have been very low. Consequently, it may safely be concluded that the short day conditions which induce the reproductive phase do not entail nitrogen shortage, as suggested by NIGHTINGALE for *Salvia*; and further, there is no experimental evidence to substantiate the hitherto commonly accepted inference that the nitrogen demand of fruiting soy beans is so great as to deplete all reserves and thereby interfere with growth.

It is also to be noted that there was a slow rate of potassium intake after flowering as compared with the increase in dry weight and nitrogen, and this was coupled with decreased succulence of the tissues. These conditions are significant and probably important factors in the cessation of growth. Nightingale, Schermerhorn, and Robbins (29) found that potassium deficiency caused an early setting of fruit, lack of growth, accumulation of carbohydrates, and a low proportion of meristematic tissue in the tomato.

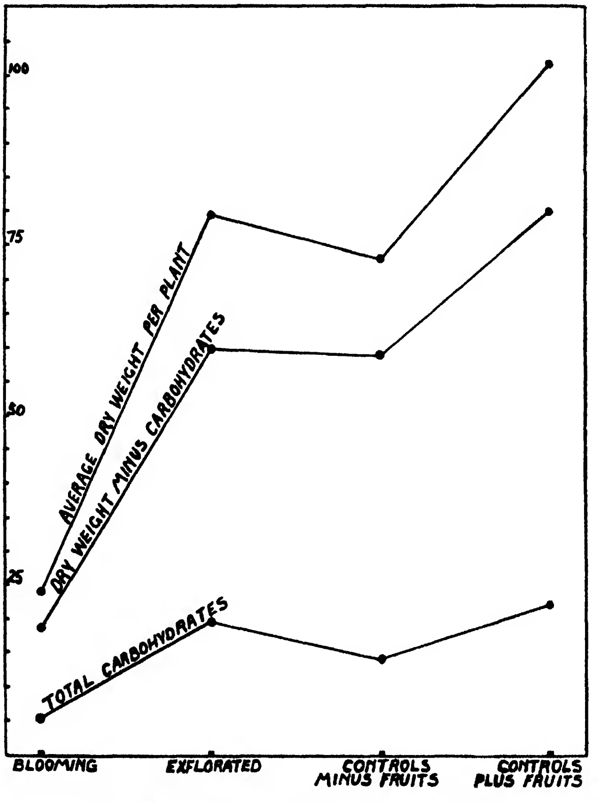

Fig. 4. Composition of soy beans; absolute weights in grams per plant.

They concluded that growth was limited because potassium is essential for synthesis of organic nitrogen from nitrates. In the case of the soy bean, however, a different interpretation is more plausible, namely, that potassium deficiency limited nitrogen assimilation only indirectly because the mechanism of cell division and growth was inhibited. The plant still had the ability to assimilate nitrogen if it could be utilized by some growing part of the plant. The controls assimilated nitrogen to supply the developing fruits, although potassium was at the same low level in the vegetative parts of the controls as in the explorated plants. In the soy bean, therefore,

potassium must be essential for the later stages of protein synthesis, *i.e.*, probably for the condensation of amino acids. Hence potassium deficiency interferes with the formation of new protoplasm and limits meristematic activity in the stems.

As in the case of nitrogen, neither calcium, magnesium, potassium, nor phosphorus accumulated in the exflorated plants and yet the development of fruits did not deplete the reserves in the controls. This means that practically the only substances stored in excess in the soy bean are carbohydrates; and that the plant, although inactive meristematically, still has the ability to take in nitrogen and minerals so that fruiting never becomes the exhaustive process which it is in some other plants (**23, 24**).

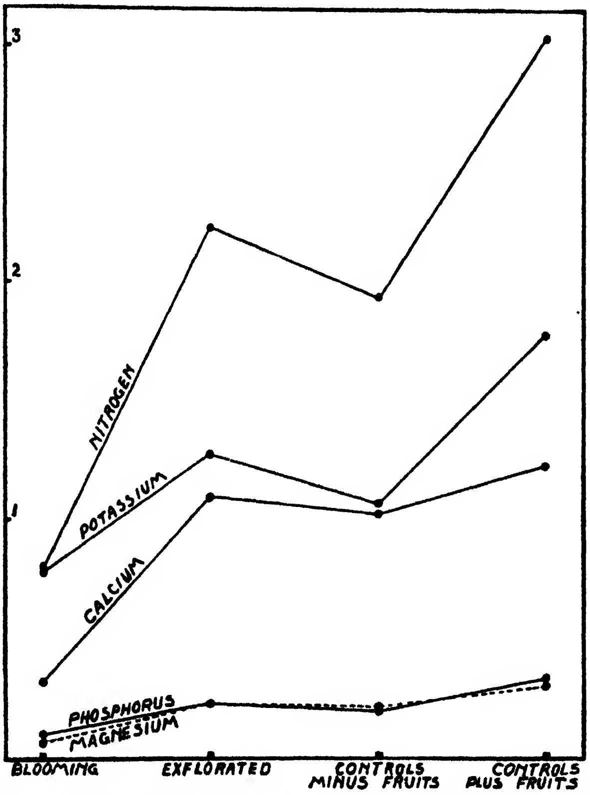

FIG. 5. Composition of soy beans; absolute weights in grams per plant.

Arguments for and against the idea of balance between carbohydrates and nitrogen as the controlling factor in growth and reproduction of plants (**1, 6, 16, 18, 19, 28, 31, 32**) must consider the behavior of photoperiodic plants (**8, 9, 10, 11**). As already mentioned, *Salvia*, a short day plant, seems to be limited in its ability to assimilate nitrogen by a short day and

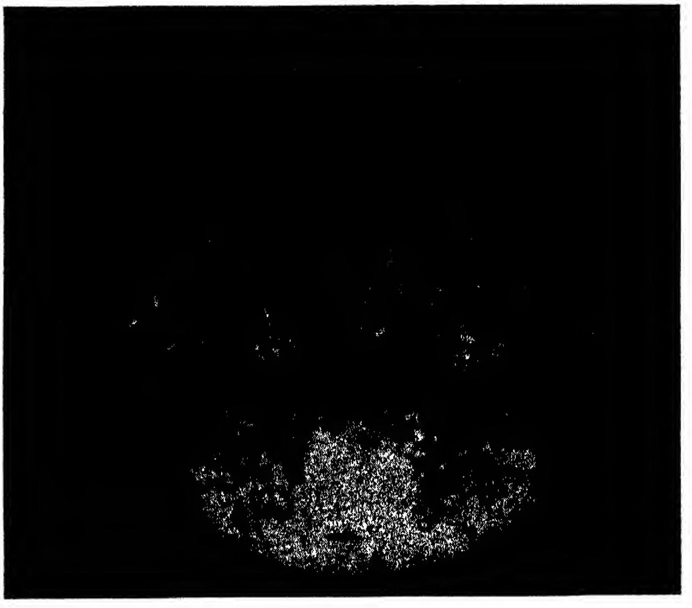

FIG. 6. Cross section of stem of exflorated soy bean plant stained with iodine to show storage of starch in medullary rays and pith.

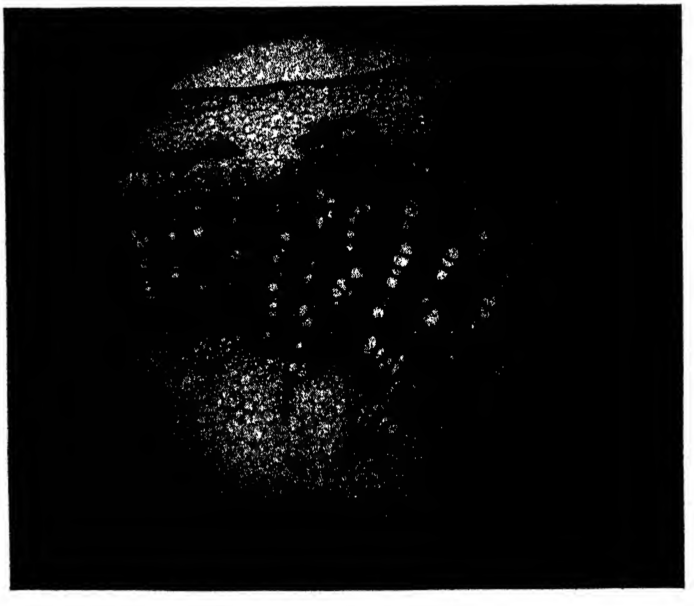

FIG. 7. Cross section of stem of normal control soy bean plant showing practically no starch (*cf.* fig. 6).

TABLE III

ANALYSIS OF 1932 ITO SAN SOY BEANS; PERCENTAGES IN TERMS OF DRY WEIGHT

Date	Plant part	Dry weight	Total nitrogen	Reducing sugars	Total sugars	Polysaccharides	Calcium	Magnesium	Phosphorus	Potassium
		%	%	%	%	%	%	%	%	%
June 6	Leaves	14.45	5.20	2.67	4.16	11.34	2.12	0.377	0.440	2.29
	Tip of stem	9.04	4.20	6.30	8.70	12.49	1.32	0.296	0.611	5.08
	Upper stem	9.38	3.20	4.47	5.78	15.09	2.14	0.377	0.296	3.65
	Lower stem	15.26	2.02	2.23	2.67	16.62	1.58	0.285	0.212	1.89
June 21	Leaves	17.77	4.56	3.27	4.13	12.89	2.02	0.356	0.378	1.76
	Tip of stem	8.58	3.76	7.91	8.57	16.54	1.05	0.235	0.621	5.70
	Upper stem	11.01	2.31	6.00	6.43	15.89	1.31	0.248	0.290	4.10
	Lower stem	17.16	1.67	3.50	3.89	17.51	1.07	0.249	0.242	2.58
July 10; blooming	Leaves	15.00	4.99	4.40	5.42	16.12	1.28	0.215	0.467	2.60
	Tip of stem	8.94	3.17	7.60	10.78	16.41	1.23	0.237	0.592	5.01
	Upper stem	10.92	2.01	6.88	8.80	21.07	1.27	0.260	0.304	4.35
	Lower stem	17.88	1.11	4.36	5.68	18.44	0.95	0.243	0.242	2.18
August 18; exflorated	Leaves	28.96	3.35	1.85	3.77	22.65	1.88	0.313	0.266	1.06
	Tip of stem	23.54	2.20	3.21	5.31	21.43	1.49	0.297	0.347	1.42
	Upper stem	27.29	1.78	2.12	4.03	24.53	0.96	0.323	0.279	0.89
	Lower stem	31.78	1.38	1.62	3.36	20.91	0.80	0.352	0.315	0.72
August 18; controls	Leaves	25.83	3.91	2.28	4.33	16.55	2.31	0.425	0.278	0.83
	Tip of stem	22.19	2.39	3.92	5.18	17.18	1.26	0.315	0.273	1.05
	Upper stem	23.56	1.68	4.03	5.39	18.27	1.04	0.394	0.310	0.81
	Lower stem	30.97	0.66	2.89	4.57	18.93	0.61	0.256	0.186	0.32
	Fruits	20.62	3.95	4.27	7.09	22.24	1.02	0.261	0.502	1.34

TABLE IV

SOY BEANS, AVERAGE TOTAL PERCENTAGES PER PLANT

TIME SAMPLED	PERCENTAGES			
June 6, seedlings (dry wt. per plant 1.30 gm.)	Dry weight Total sugars Polysaccharides Total nitrogen	12.94 4.46 12.30 4.62	Calcium Magnesium Phosphorus Potassium	2.01 0.362 0.416 2.56
June 21, vegetative (dry wt. per plant 7.61 gm.)	Dry weight Total sugars Polysaccharides Total nitrogen	15.79 4.56 14.24 3.81	Calcium Magnesium Phosphorus Potassium	1.72 0.318 0.356 2.36
July 10, blooming (dry wt. per plant 21.57 gm.)	Dry weight Total sugars Polysaccharides Total nitrogen	14.18 6.34 17.65 3.35	Calcium Magnesium Phosphorus Potassium	1.19 0.231 0.384 2.92
August 18, exflorated	Dry weight Total sugars Polysaccharides Total nitrogen	28.80 3.82 22.54 2.61	Calcium Magnesium Phosphorus Potassium	1.47 0.322 0.282 0.98
August 18, controls (minus fruits)	Dry weight Total sugars Polysaccharides Total nitrogen	25.84 4.65 17.40 2.73	Calcium Magnesium Phosphorus Potassium	1.65 0.379 0.267 0.744
August 18, controls (plus fruits)	Dry weight Total sugars Polysaccharides Total nitrogen	24.68 5.11 18.30 2.96	Calcium Magnesium Phosphorus Potassium	1.54 0.358 0.352 0.855

flowering accompanies the subsequent accumulation of carbohydrates. The experimental evidence for this conclusion, however, is very meager. In some long day plants the greatest accumulation of carbohydrates occurs under short day conditions, but flowering is initiated only by long days (**35**, **36**).

From the results of ringing and defoliating experiments, HARVEY (**16**) claims that the vegetative responses of apple shoots are very definitely controlled by the ratio of carbohydrates to nitrogen. ROBERTS (**33**) and THOMAS (**34**) suggest that accumulation of carbohydrates interferes with nitrogen metabolism and retards growth. On the other hand, a high ratio of carbohydrates to nitrogen seems to be the result of inhibited or retarded growth and not the cause of it in many cases. HARTWELL (**15**) found that

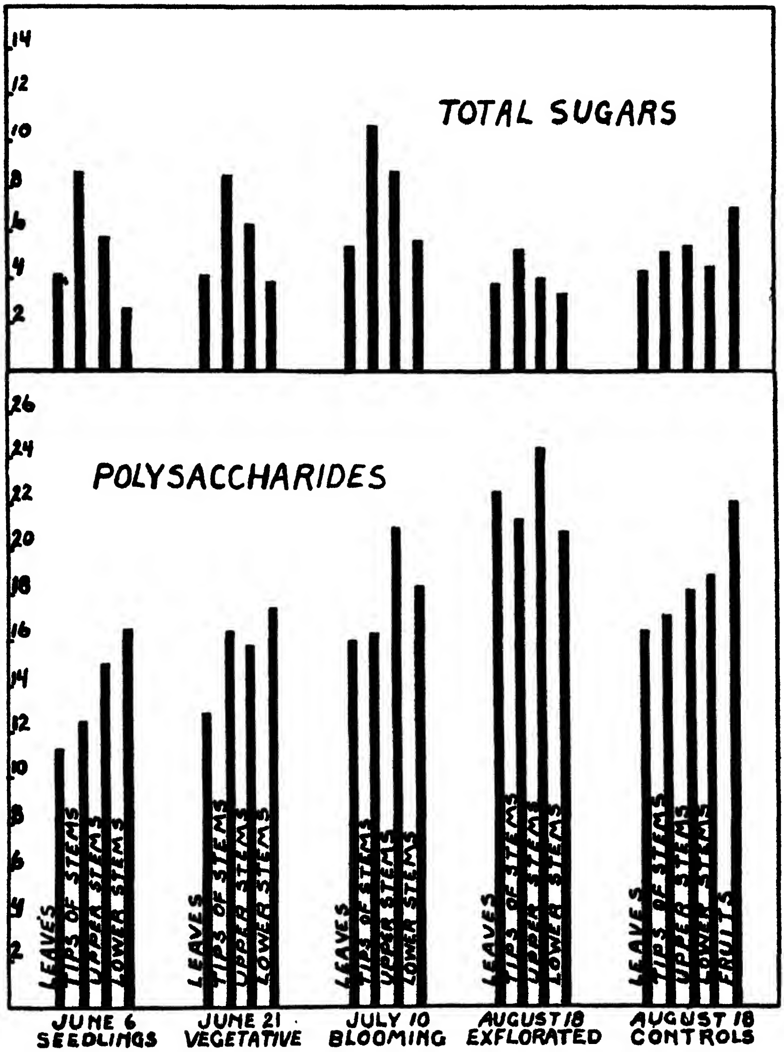

Fig. 8. Composition of soy beans; percentages in terms of dry weight.

accumulation of starch seemed to be correlated in general with conditions which caused a retardation of growth. If high carbohydrates limit growth, we still lack convincing experimental data to explain why long days stimulate growth in plants which have become high in carbohydrates under the influence of short days. This happens in some long day plants (**35**, **36**), and Garner and Allard (**8**, **9**) have rejuvenated soy beans and other short day plants after they had begun to set fruits by subjecting them to long days.

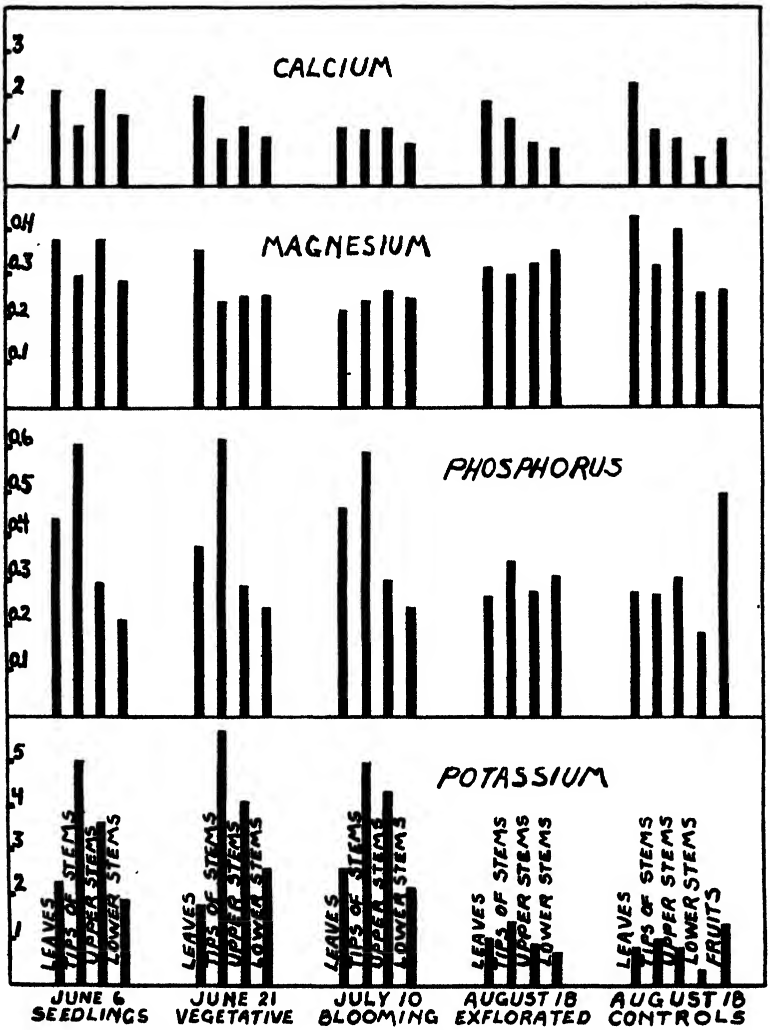

FIG. 9. Composition of soy beans; percentages in terms of dry weight.

The data for 1932 (tables III and IV and figs. 8–10) are given in full, although this involves some duplication, because they cover a full life cycle and because they represent a more detailed analysis of the plants than do those for 1931. These data disclose a high potassium content associated with high moisture content during the period of rapid growth, but nitrogen was not much higher in vegetative than in mature plants. Cessation of growth could hardly be attributed to a shortage of nitrogen, but the shortage of potassium probably had something to do with the decrease in mois-

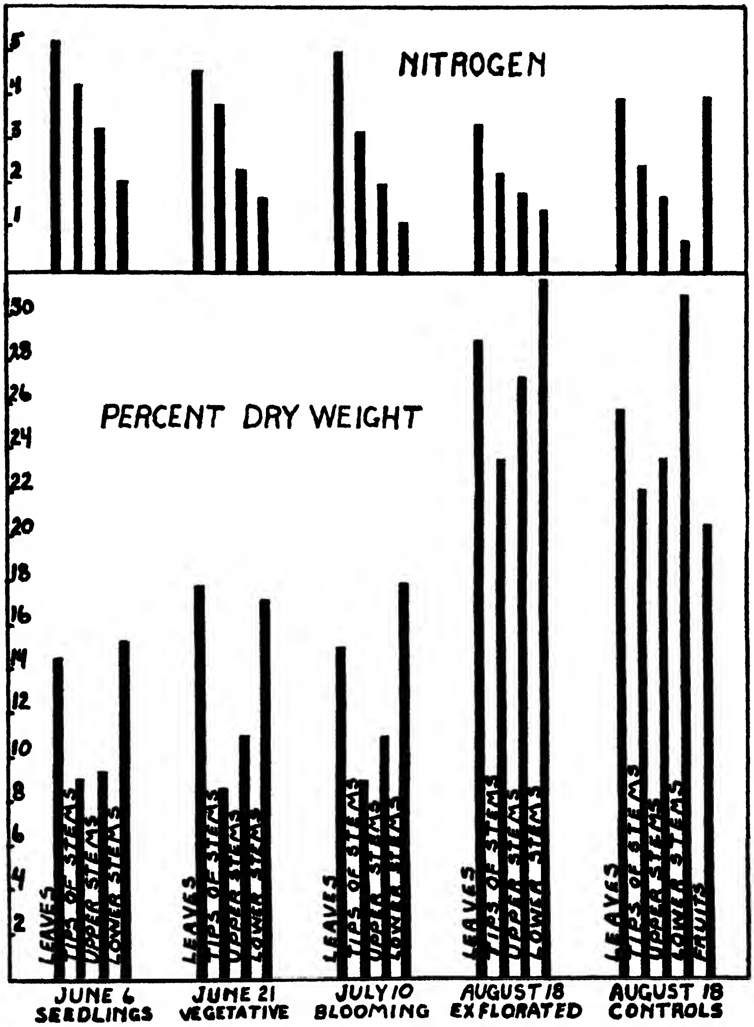

FIG. 10. Composition of soy beans; percentages of nitrogen in terms of dry weight and percentage dry weight in terms of fresh weight.

ture content and the retarded growth. Physiological dryness of tissues seems to be characteristic of plants deficient in potassium (**36, 38**). The appearance and behavior of exflorated soy bean plants are similar to those described by JANSSEN and BARTHOLOMEW (**17**) for tomato plants grown in a medium lacking in potassium. The percentages of phosphorus are highest in regions of rapid growth, stem tips of vegetative plants and fruits of control plants, but it does not decrease in other parts of the plant so much as does potassium when the plant stops growing.

Decreases in the percentages of potassium and moisture in the stems and leaves of mature soy bean plants are not due to fruiting since they are not prevented by exfloration. Likewise exfloration does not prevent cessation of growth. It seems likely then that the moisture, potassium, and to a less extent the phosphorus contents of the tissues are in some way related to nitrogen metabolism and the growth of new tissues. Whether these represent causes or only conditions accompanying particular growth responses cannot be stated with certainty.

In conclusion it may be stated that although growth of the soy bean plant normally stops at about the time that the fruits develop, plants from which the flowers are removed do not grow any larger than such normal plants. Hence cessation of growth is not due to fruiting, since it is not prevented by exfloration. Neither is it to be explained as a result of limited ability of the plant to absorb nitrogen. The best available explanation at present seems to be that the meristematic regions of the stem become inactive or dormant owing apparently to the decrease in day length, and the cessation of nitrogen absorption and the accumulation of carbohydrates are due to lack of growth. The exact mechanism of the action of the photoperiod is unknown, but in the soy bean it is related in some way to the moisture and potassium contents of the tissues. Explorated soy bean plants show many of the symptoms of potassium starvation.

Summary

1. In normal soy bean plants growth stopped at about the time that the fruits developed, which would suggest that fruit development was responsible for the cessation of growth.

2. Exfloration did not increase the vegetative development, growth ceasing simultaneously in explorated and control plants.

3. The soy bean is a photoperiodic plant and apparently the shortening of the day length not only initiates the reproductive phase but also curtails the vegetative processes.

4. A shorter day is required to inhibit growth in the soy bean than is necessary to initiate flowering.

5. There was an abnormal accumulation of carbohydrates in explorated plants.

6. Nitrogen did not accumulate in explorated plants, but on the other hand it was not depleted in control plants by the development of fruits.

7. Neither calcium, magnesium, phosphorus, nor potassium was depleted in control plants by the development of fruits.

8. As the plants ceased to grow there was an increase in the percentage of dry weight, or in other words a decrease in moisture content, in both explorated and control plants.

9. This decrease in moisture content and cessation of growth were associated with a marked decrease in the percentages of potassium in all parts of the stems and the leaves. Phosphorus decreased in the stem tips when the stems ceased to elongate.

10. The behavior of exflorated soy bean plants resembles the responses of some other plants to potassium starvation, and the suggestion is therefore made that the length of day may affect vegetative growth through its influence on the concentration of potassium in the tissues of the plant.

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LIMITATIONS OF BLACKMAN'S LAW OF LIMITING FACTORS AND HARDER'S CONCEPT OF RELATIVE MINIMUM AS APPLIED TO PHOTOSYNTHESIS

B. N. SINGH AND K. N. LAL

(WITH NINE FIGURES)

Problem and mode of attack

The universality of the photosynthetic process and its fundamental nature and importance have long been recognized, and little is known regarding the systems involved and their exact working in consonance with both external and internal factors. From the time it came to be recognized that CO_2 was absorbed by the leaves during photosynthesis and that with an increase in its concentration the rate of assimilation increased, attempts have been made to determine an optimum concentration of carbon dioxide, a percentage which may induce rapid photosynthesis, leading to increased growth in plants.

With the formulation of LIEBIG's law of the minimum (7) and with the advance in our knowledge of the subject of photosynthesis, it came to be realized that the process is conditioned by numerous factors. PFEFFER (12) and PANTANELLI (13) realized that the optimum value in photosynthesis was not fixed under the influence of any single factor.

Considerable work has been carried out in recent years on the relationship of photosynthesis to external factors, and several laws indicating the possible relationship between the external factors on the one hand and carbon assimilation on the other have been evolved.

BLACKMAN (1) first called attention to the fact that in a phenomenon such as photosynthesis, the focusing of attention on a single factor in disregard to the influence of others leads to erroneous results. A study of the interrelation of the conditioning factors led him to formulate the principle of limiting factors, which he has stated in the following axiom: "When a process is conditioned as to its rapidity by a number of separate factors the rate of the process is limited by the pace of the slowest factor." This theory stimulated investigations designed to test the validity of BLACKMAN's proposals.

His observations were confirmed by MATTHAEI (11), BLACKMAN and SMITH (2), and WILMOTT (16), but later workers like HOOKER (5), BROWN (4), BOYSEN JENSEN (3), and HARDER (6) criticized the theory and showed that the law of limiting factors was not infallible. This theory was subsequently modified by HARDER (6) in his concept of relative minimum, but at present both theories seem to be equally supported on an experimental

basis. One of the vital points of difference between the viewpoints of BLACKMAN and HARDER is that while the former holds that there is a sudden change in direction of the ascending curve to a horizontal one when the limiting factor comes into operation, the latter holds that the curves are extremely regular and that there is no sudden change of the ascending curve to a horizontal phase. Moreover, the rate is not governed by one factor and that alone, as was supposed by BLACKMAN, but is also conditioned by the intensity of others present in relative minimum. Both these observations are equally supported by experimental data and the question therefore remains, Is there a sharp break in the curve or is it smooth in form?

The present work is the outcome of a study concerned with the nature of growth in relation to external and internal factors. In trying to study growth with respect to other metabolic activities, more particularly assimilation and respiration, it was necessary to determine under laboratory conditions the relation between CO_2 assimilation and such external factors as temperature, light, and carbon dioxide. Special effort has been made to estimate their effect on the rate of photosynthesis when they are present in concentrations and intensities in which they naturally occur in the field.

The results have thrown considerable light on the nature as well as on the applicability of the law of limiting factors and the theory of relative minimum, and these have therefore received separate treatment in the present paper.

Investigation

MATERIAL.—Material was selected from crops of economic importance such as wheat (var. Pusa 4), linseed (var. 1150 S), and sugar cane (var. Reori) grown both under natural environment and under conditions of optimum nutritional supply at the Experimental Farm of this Research Station.

APPARATUS.—The apparatus consists of a carbon dioxide absorbing set, a CO_2 generating tower, a constant temperature bath, and a commutator and clockwork arrangement identical in most respects with the one designed by BLACKMAN for investigations on vegetable assimilation. The plant chamber is of special construction, with inlet and exit tubes, an opening at the top for the insertion of a thermometer, and a depression for the attachment of a small funnel containing water into which the leaf base is dipped. The front and back sides of the chamber are of glass, one pane of which can easily be removed for admitting the experimental leaf.

Half-watt 220-volt Phillips bulbs of various intensities were used as the source of illumination. The exact intensity in each case was determined by comparison with standard bulbs and the values expressed in candle

power. A screen of running water intervened between the bulb and the plant chamber in order to prevent heat from the bulb reaching the bath.

The temperature of the bath was maintained constant for each series of experiments by means of thermo-gas regulators. The experimental temperature varied between 23° and 40° C., and never fluctuated more than ± 0.05° C.

The CO_2 concentration used was between 0.0 and 0.5 per cent., according to the requirement of the experiment. The percentage of carbon dioxide by volume was calculated by the formula:

$$\text{percentage } CO_2 = \frac{\text{wt. of gas in control tubes per hr. in gm.} \times 500}{1500} \times 100,$$

where 500 is the approximate volume of the gas weighing 1 gm. and 1500 the speed of dropping in the aspirators per hour.

The details of temperature, light intensity, and CO_2 concentration in a particular experiment are given in the tables.

Experimental procedure

The general procedure consisted in the selection of mature healthy normal green leaves from an average plant of known age a day previous to the time of the experiment. These leaves were kept near a north window, covered with an open belljar with their ends in water and left over night to attain laboratory conditions. The leaves were regularly collected at 4 P. M. after a period of rapid assimilation and the experiment was started each day at about 10 A. M. after recording the fresh weight and the area of the assimilating leaf surface.

The leaves prepared in this manner were arranged in the assimilation chamber and connections were then made to the CO_2 generating set and the commutator. A current of air containing a definite amount of carbon dioxide was divided into two equal parts. One was passed over the experimental leaf and was then withdrawn through a Pettenkoffer tube containing a measured quantity of standard baryta solution. The other part was passed directly through another Pettenkoffer tube containing the same amount of barium hydroxide. After a definite interval, the two tubes were disconnected, their contents poured into separate beakers, and the solution titrated against standard hydrochloric acid. The difference in the acid value of the two solutions is due to the reduction in the amount of CO_2 absorbed by the assimilating leaf. A preliminary tube was always run for 1.5 hours before taking the actual readings, followed by three consecutive hourly readings for the determination of the apparent assimilation. The respiration of the same material under identical conditions, but in complete darkness, was determined and the real assimilation calculated for 100 sq. cm. of assimilating area per hour. For each experiment a new leaf was used.

Care was taken to determine experimentally the amount of external carbon dioxide absorbed by the solution during the course of filling, fixing, and titrating the solution. This "washing factor" was determined several times during the course of the experiment and due allowance made for it in the readings.

Precaution was also taken to avoid as far as possible the resistances in the diffusion and photochemical phases of the process (8, 9), or to bring them to a common factor throughout the whole series. Variations due to season were avoided by performing the experiment in a single season of the year. The observations were taken at definite hours of the day and under definite artificial illumination, thus reducing variation in resistance due to time of day, intensity of illumination, and time of beginning of the experiment. To avoid the effect of previous history of the leaf only healthy leaves from average selected plants were taken and kept with the leaf base in water for several hours to equalize moisture conditions. Only leaves of the same developmental stage were used.

The course of assimilation at various concentrations and combinations of external factors was followed with only the mature leaves during the adolescent phase of the life cycle.

Results

The data are mainly confined to three of the most important external factors, light, temperature, and carbon dioxide, which have been shown by previous workers to have considerable influence on the rate of photosynthesis.

CO_2 CONCENTRATION AND ASSIMILATION

With the preliminary precautions already described, observations were made on the effect of carbon dioxide on the rate of assimilation. Concentrations both below and above that present in the field were used, the former being maintained by absorbing some of the CO_2 in the current by potash and the latter by providing extra quantities of this gas in the stream by the CO_2 generating tower.

Since the experiments were conducted entirely under laboratory conditions, other factors like light intensity and temperature were also controlled. Under a constant temperature and a constant light intensity, the CO_2 assimilation was determined at 0.02 per cent. external concentration of carbon dioxide. The results are set forth in table I, experiment 1.

Under the conditions of the experiment, it is evident that the green leaf is not in a position to obtain any appreciable quantity of carbon dioxide from its surroundings. It has naturally to depend on the CO_2 evolved during respiration for the synthesis of carbohydrates. The quantity of

TABLE I

EFFECT OF CO_2 CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.
ILLUMINATION, 90 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO_2 CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	*mg.*
July 1	1	0.02	0.403
" 2	2	0.051	1.050
" 3	3	0.0806	1.150
" 4	4	0.1280	1.005
" 5	5	0.3740	-0.960

respiratory carbon dioxide for the entire leaf area being small, the values of assimilation are naturally low.

The carbon assimilation under slightly greater concentration of carbon dioxide, *viz.*, equivalent to that of the atmosphere, was then determined. The results are shown in table I, experiment 2. In this case assimilation is higher than the preceding, indicating that with increase in the percentage of carbon dioxide the value of assimilation increased.

To test whether an increase in assimilation will follow a further increase in the percentage of carbon dioxide, the concentration was raised to 0.0806 per cent. Experiment 3 indicates that the intensity of assimilation does increase.

Experiments 4 and 5 were designed to study the effect of still higher concentrations of carbon dioxide on the rate of photosynthesis. Light intensity and temperature being kept the same as before, the variations found in the readings should be due to the increase in the concentration of carbon dioxide.

The rates of assimilation obtained under different concentrations of carbon dioxide (table I) show that up to 0.08 per cent. of carbon dioxide the velocity of the reaction increases, and any further increase is accompanied by a decrease in the intensity which finally attains a negative value under 0.374 per cent. It would thus follow that after 0.08 per cent. carbon dioxide concentration any extra quantity of carbon dioxide produces some sort of inhibitory effect on the process, the intensity of inhibition increasing with an increase in its concentration.

It has just been shown that assimilation under the particular temperature and light intensity is highest at 0.08 per cent., the readings falling

gradually on both sides of this maximum. Why should the intensity of photosynthesis rise for some time and then decline with increasing concentration of carbon dioxide? In the light of BLACKMAN's concept we can explain it as due to the fact that the carbon dioxide supply up to this stage is limiting; hence an increase in the limiting factor, the pace of the slowest factor, increases the rate of assimilation. Following HARDER, the same phenomenon may be explained on the basis that the carbon dioxide is at first lesser in quantity or in relative minimum than other conditioning factors which are exerting greater influence over the process. And as its concentration is increased the intensity becomes still greater, finally equaling that of other factors in which the readings are maximum. The subsequent fall with increase in CO_2 concentration may be due to the deleterious effect of excessive carbon dioxide.

Whatever explanation we may give to the nature of the phenomenon, it is clear from the data obtained (fig. 1, 90 c.p.) that with increasing concentration of CO_2 the intensity of photosynthesis increases in regular sequence. The sudden transference of the ascending phase into a horizontal one, as was observed by BLACKMAN and his co-workers, is not apparent in the present case. Instead the curve shows a gradual rounding, thus probably answering one of the vital questions raised in our introduction. Further, the stationary phase when the limiting factor comes into operation as observed by BLACKMAN is not marked in the present case. If it is present at all, it may be found only within a small range of concentration. The decline phase on the other hand is characteristically evident.

In order to test the validity of these results obtained for sugar cane, inquiry along strictly similar lines was conducted on wheat and flax plants grown side by side under similar conditions. The gradations of the carbon

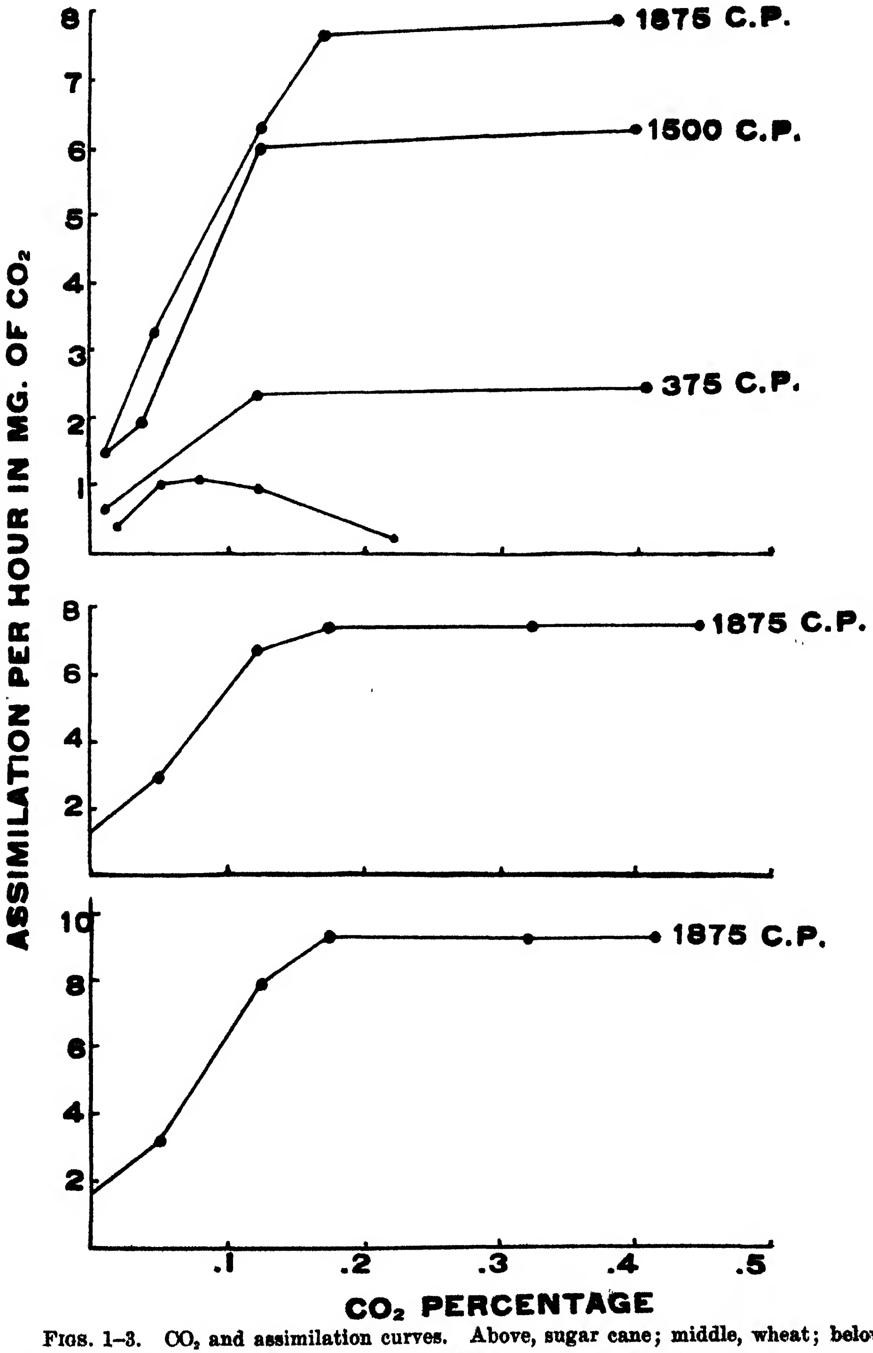
TABLE II

EFFECT OF CO_2 CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF WHEAT, PUSA 4

TEMPERATURE, 30.0° C.

ILLUMINATION 1875 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO_2 CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	*mg.*
March 11	22	0.05	2.82
" 11	29	0.124	6.75
" 12	30	0.172	7.37
" 13	31	0.320	7.40



FIGS. 1–3. CO_2 and assimilation curves. Above, sugar cane; middle, wheat; below, flax. Illumination in candle power. Lowest curve for sugar cane, 90 c.p.

dioxide range as well as the constancy of temperature and light intensity were maintained, although the magnitude of each was purposely raised in order to compare our data at higher intensities of the factors.

In spite of material difference in the leaves which were gathered from plants of three different types, curves (figs. 2, 3) differing only in magnitude but essentially similar in nature to the one obtained for sugar cane were secured in these two cases as well (tables II and III).

TABLE III

EFFECT OF CO_2 CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF LINSEED, 1150 S

TEMPERATURE, 30.0° C.
ILLUMINATION, 1875 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE	EXPERIMENT NO.	CO_2 CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	*mg.*
March 8, 1932	25	0.05	3.24
February 1, 1933	26	0.124	7.94
" 2, 1933	27	0.172	9.23
February 3, 1933	28	0.32	9.23

Medium and high intensities of light were also used on sugar cane to test the nature of the reaction as affected by increased illumination. Table IV includes three experiments with medium light intensity.

TABLE IV

EFFECT OF CO_2 CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.
ILLUMINATION, 375 C.P. HALF-WATT PHILLIPS BULB AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO_2 CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	*mg.*
May 7	6	0.011	0.680
" 8	7	0.121	2.391
" 9	8	0.407	2.510

The readings (table IV) show an initial rapid increase in the rate of assimilation, followed by a more or less stationary phase. In this case

there is a wider range in assimilation than in the previous case. The intensity of the reaction is greater in the present case, however, because of the greater supply of energy now available.

A series of experiments was performed also with higher light intensity. In this series, as is evident from the curves shown in figure 1 and the values in tables V and VI, the nature of the reaction as judged by the rate of assimilation remains the same, differing only in intensity and magnitude.

TABLE V

EFFECT OF CO_2 CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 1500 C.P., HALF-WATT PHILLIPS BULBS AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO_2 CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	*mg.*
July 12	10	0.011	1.48
" 11	9	0.036	1.964
" 13	11	0.124	6.100
" 18	12	0.403	6.38

TABLE VI

EFFECT OF CO_2 CONCENTRATION ON RATE OF CARBON ASSIMILATION BY MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

ILLUMINATION, 1875 C.P., HALF-WATT PHILLIPS BULBS AT 16 CM. DISTANCE

DATE (1932)	EXPERIMENT NO.	CO_2 CONCENTRATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		%	*mg.*
July 19 ..	13	0.0476	3.303
" 20 .	14	0.124	6.290
" 21	15	0.172	7.770
" 22	16	0.388	7.920

It is thus seen (table VI) that a higher light intensity increases the rate of photosynthesis several-fold. The higher rate is possibly correlated with the greater supply of available energy, and in fact it was not practicable within the limited time at our disposal actually to determine the concentra-

tions at which the toxic or inhibitory effect comes into prominence, since the leaves could endure sufficiently high CO_2 concentration, up to ten times higher than that in the field, under very high intensities. Several readings were not possible for each light intensity since the investigations necessarily had to be finished within a limited stage in the life cycle of crops in order to avoid the effect of age and developmental stage on assimilation.

The curves of figure 1 indicate that with the higher intensities of light, the ascending phase becomes more and more characteristic and prominent. The magnitude of the ascending phase in contradistinction to the level phase is greater as we use higher light intensities, and it may therefore be expected that if an unlimited supply of light energy be available, together with a continuous and increasing supply of carbon dioxide, the inflexion phase may be entirely lost, the reaction proceeding in a linear fashion. But how far such a type of curve can be expected in practice is a matter for experimentation. It is just possible that, even with all the external factors supplied at maximum level, the nature of the reaction may remain the same on account of the control of the process by physiological or internal factors, namely, the specificity of the plant, the nature of the products formed, the chlorophyll apparatus, the water content, etc.

Light intensity and assimilation

That light is essential for photosynthesis is well known, but owing to the complexities introduced by the simultaneous influence of other factors, carbon dioxide and temperature, it becomes difficult to gauge the relationship at its correct value. For example, as already shown, the influence of carbon dioxide on the rate of assimilation is found to be dependent upon light, the rate rising with increased intensity. It is also known that temperature influences the rate, a rise being obtained by raising the temperature until a certain degree is reached followed by a decline with further rise in temperature. These facts lead one to conclude that in investigating the influence of light, the simultaneous influence of two other factors, carbon dioxide and temperature, must be taken into consideration.

In the present study, which does not aim at a detailed investigation of the problem but is simply concerned with the determination of the light intensity required to deplete the normal concentration of carbon dioxide, the effect of increasing light intensity on the rate of photosynthesis has been tested at four different concentrations of CO_2 both below and above that normally present in air.

Zero percentage external CO_2 concentration.—Under such a condition the plant is not in a position to utilize any other CO_2 except that which it is itself evolving during the downgrade process of respiration. The readings obtained under such conditions are given in table VII.

TABLE VII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.

CO_2 CONCENTRATION, 0.0 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		c.p.	*mg.*
July 1 .	1	90	0.403
" 7 ...	6	375	0.680
" 12	10	1500	1.480
" 13 .	40	1875	1.480

It is seen that the rates of assimilation at 90, 375, 1500, and 1875 c.p. are 0.4, 0.68, 1.48, and 1.48 mg. respectively per hour. This shows that the respiratory carbon dioxide, which is the only carbon dioxide available for photosynthesis under such circumstances, is not all used at lower light intensities. Experiments 1 and 6, dealing with 90 and 375 c.p. respectively, show that under these light intensities the plant is not in a position to synthesize what it respires. As the light intensity is increased the photosynthetic value rises, and ultimately the leaf is able to utilize the total output of carbon dioxide (experiment 10). When the two processes balance each other, *i.e.*, when the compensation point is reached, no more increase in the readings is obtained with further increase in light intensities.

What is the nature of the reaction at this stage, namely, how far does the photosynthetic chain of reactions proceed and how are they correlated with the opposite series of reactions connected with the respiratory process? Answers to these questions may throw light on the nature of the two interrelated reactions.

ATMOSPHERIC CONCENTRATIONS OF CO_2.—Since all the plants grew under this concentration it was thought advisable to investigate the effect of increasing illumination on the rate of photosynthesis under such atmospheric percentage of CO_2 in the case of different plants. Besides the sugar cane leaves which were subjected to detailed investigation, leaves of wheat and flax were experimented upon also.

The readings obtained for sugar cane leaves under this concentration and different light intensities are shown in table VIII.

It is evident that with higher light intensities (90 to 1875 c.p.) the rate of assimilation increases from 1.05 to 2.23 mg. Even at the high light intensity of 1500 c.p. the readings show an increase when the intensity is

TABLE VIII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.
CONCENTRATION, 0.05 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		c.p.	*mg.*
July 2, 1932	2	90	1.05
March 6, 1933	17	375	1.52
" 7, 1933	18	1500	2.1
" 8, 1933	19	1875	2.23

increased to 1875 c.p. The constant phase of the activity could not be determined on account of lack of higher light intensities. The rates of assimilation of wheat leaves under the different light intensities and atmospheric concentration of CO_2 are shown in table IX.

TABLE IX

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF WHEAT, PUSA 4

TEMPERATURE, 30.0° C.
CO_2 CONCENTRATION, 0.05 PER CENT.

DATE (1933)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		c.p.	*mg.*
March 9	20	375	2.05
" 10	21	1500	2.74
" 11	22	1875	2.82

On comparing the rates (table IX), it is clear that the results with wheat are rather similar, the rate rising with the light intensity. The readings at 1500 and 1875 c.p. are 2.74 and 2.82 mg. There is not much difference between the two, however, and we can say that 1875 c.p. is sufficient to deplete all the carbon dioxide normally present in the atmosphere.

Linseed plants showed contrasting results. The readings at 375, 1500, and 1875 c.p. are shown in table X. The rate of assimilation does not rise with increase in light intensity beyond 1500 c.p.

TABLE X

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF LINSEED, 1150 S

TEMPERATURE, 30.0° C.
CO_2 CONCENTRATION, 0.05 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		c.p.	*mg.*
March 16	23	375	2.72
" 17	24	1500	3.24
" 18	25	1875	3.24

From the results obtained with regard to the influence of light on the photosynthetic rate of the three crop plants, it is seen that while light intensity of 1875 c.p. and more is necessary for maximal values in the cases of wheat and sugar cane, an intensity of 1500 is sufficient for linseed. In other words, linseed plants under normal conditions of CO_2 require less light intensity for attaining their optimum value than do sugar cane and wheat. Whether such differences are in any way connected with the nature of the end product of the upgrade process (sugar in sugar cane, starch in wheat, and oil in linseed) is a problem requiring further investigation.

MEDIUM CO_2 CONCENTRATION.—Only the sugar cane leaves were the subject of experimentation under medium concentration of carbon dioxide. The rates of assimilation are shown in table XI.

TABLE XI

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

TEMPERATURE, 30.1° C.
CO_2 CONCENTRATION, 0.12 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		c.p.	*mg.*
July 4 ...	4	90	1.005
" 8	7	375	2.391
" 13	11	1500	6.10
" 20	14	1875	6.29

There is a clear and characteristic rise similar to that observed under normal concentrations above, but differing in magnitude. Under such a concentration the rise is more or less proportional to light intensity (fig. 4). The stationary phase could not be secured, owing to the lack of sufficiently strong light which we had not at our disposal when the investigations were in progress.

OPTIMUM CO_2 CONCENTRATION.—In this case the values as shown in table XII indicate the rate of photosynthesis of sugar cane leaves.

TABLE XII

EFFECT OF LIGHT INTENSITY ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF SUGAR CANE, VAR. REORI

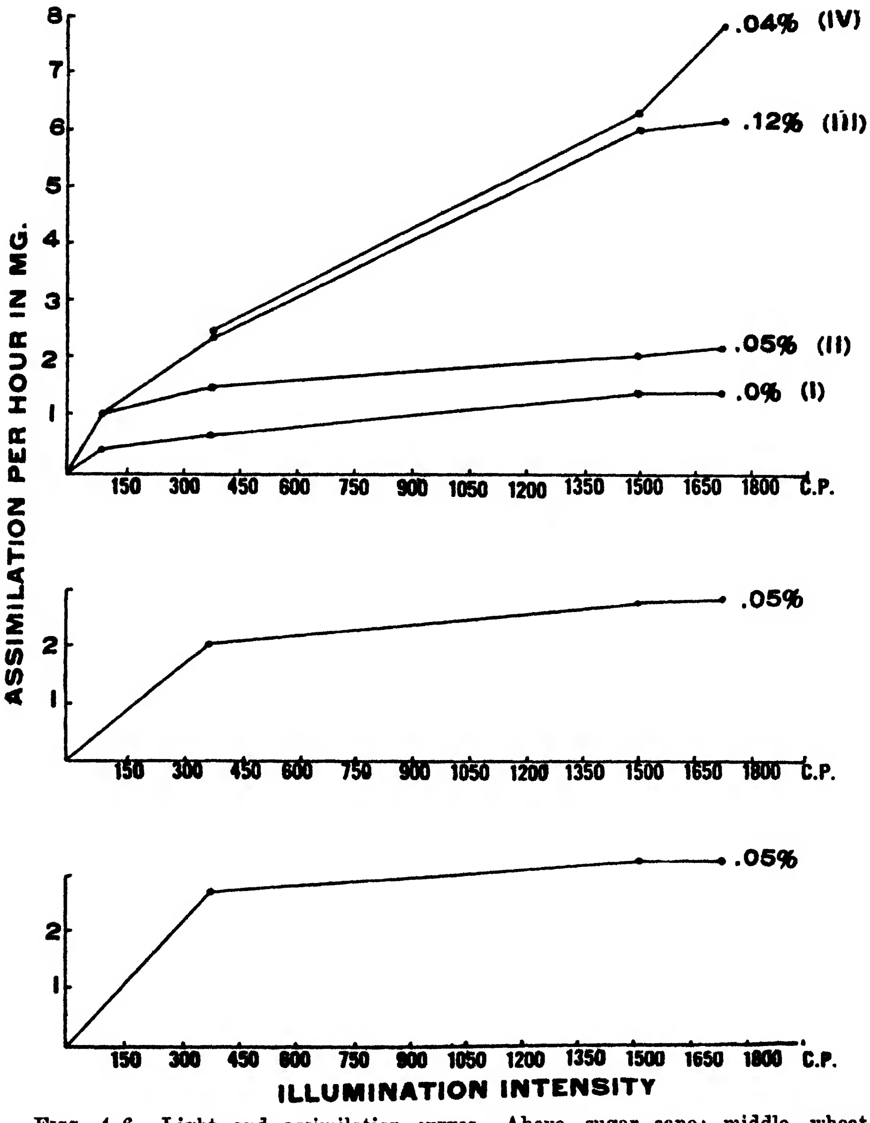
TEMPERATURE, 30.1° C.

CO_2 CONCENTRATION, 0.4 PER CENT.

DATE (1932)	EXPERIMENT NO.	ILLUMINATION	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		c.p.	*mg.*
July 5	5	90	−0.96
" 9	8	375	2.51
" 18	12	1500	6.38
" 22	16	1875	7.92

The curves shown in figure 4 display a direct proportionality between light intensity and the rate of assimilation from 375 c.p. onward, showing that light governs the reaction under conditions of optimum CO_2 concentration. At 90 c.p. the intensity of light is too feeble for this high concentration of CO_2, under which circumstances the carbon dioxide exerts its toxic effect as shown by negative photosynthesis.

The nature of the reaction as measured by the CO_2 intake under different concentrations of CO_2 at different light intensities (fig. 4) shows at first an ascending phase but the steepness of the curve depends upon the concentration of carbon dioxide. The richer the environment the higher is the angle of curvature and the higher the values. In the case of a zero percentage CO_2 concentration the curves become flattened when light intensity is raised from 1500 to 1875 c.p., showing that under these conditions CO_2 is limiting the rate. But such a stationary phase was not observed at 1500 and 1875 c.p. in the case of higher concentrations. This shows that under higher concentrations of carbon dioxide the existence of the stationary phase requires very high light intensity, a theory which has still to be experimentally supported.



FIGS. 4–6. Light and assimilation curves. Above, sugar cane; middle, wheat; below, flax.

In the light of BLACKMAN's theory, curve no. 1 (fig. 4) can be explained as consisting of two parts, an ascending phase when light is limiting and a stationary one when CO_2 is limiting. In the case of other curves we have only the ascending phase, indicating a stage when light is always limiting. The curves can equally be explained on the basis that during the ascending

phase the light intensity is in relative minimum and during the stationary phase the other factor, CO_2, falls short of the requirement.

We shall discuss critically the relative importance of the theory of limiting factors and that of relative minimum after we have dealt with the third factor, namely, temperature.

Temperature and assimilation

Under ordinary conditions in the field, the CO_2 concentration during the day does not increase to a great extent. The values are more or less stationary, but so far as the temperature is concerned it shows decidedly wide fluctuations. In order to determine the optimum temperature under normal concentration of carbon dioxide, a series of experiments as shown in table XIII was performed.

TABLE XIII

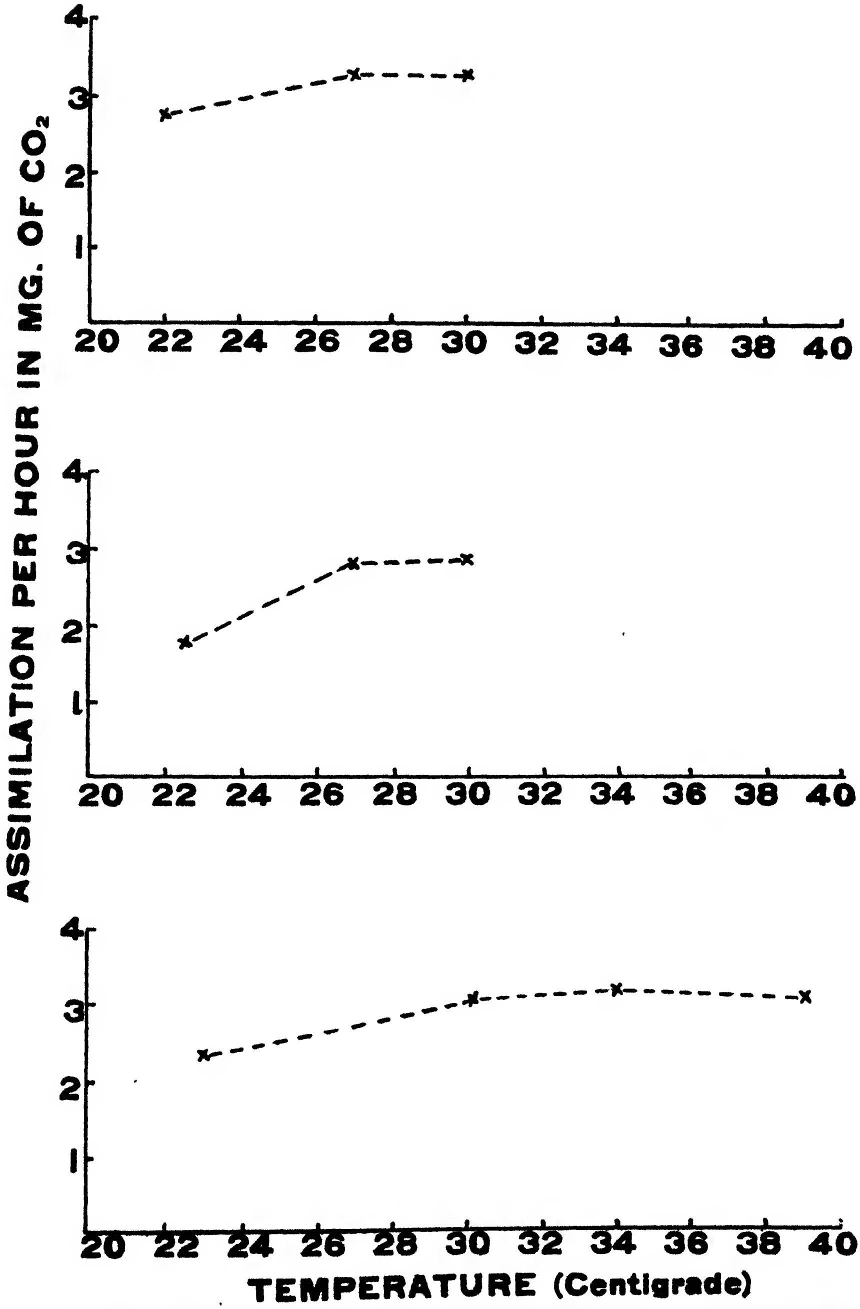
Effect of temperature on rate of carbon assimilation of mature leaves of sugar cane, var. Reori

CO_2 concentration, 0.05 per cent.
Light intensity, 1875 c.p.

Date (1932)	Experiment no.	Temperature	Real assimilation per 100 sq. cm. per hr.
		°C.	mg.
July 19, 1932	13	30.1	3.03
March 9, 1933	36	23.0	2.3
" 11, "	38	34.0	3.2
" 12, "	39	39.0	3.05

Only four temperatures, which came within the purview of fluctuations within these regions and to which the plants are subjected at this time of the year, were selected: 23°, 30°, 34°, and 39° C., it being considered unnecessary to go above or below these temperatures. In each case light intensity sufficiently high so as not to limit the rate was used. The concentration of carbon dioxide was the same as that obtained in the field.

From the values shown (figs. 7–9), it is clear that the rate of assimilation rises with rise in temperature for some time, followed by a decline. Two influences seem to be at work: first, the effect of temperature in raising the amount of assimilation of which the leaf is capable; second, an injurious effect which becomes more and more prominent with high temperature. The rate at any point is therefore the result of an accelerating as well as of a depressing effect of temperature. But at higher temperatures a



FIGS. 7–9. Temperature and assimilation curves. Above, flax; middle, wheat; below, sugar cane.

point is reached when the acceleration due to increased temperature is balanced by the depressing action. This point gives the maximum assimilation and lies for sugar cane leaves near 34° C.; but, as may be seen from the individual readings in experiment 38, the initial value is not maintained very long.

At temperatures higher than 34° C. the injurious effect gains ascendency and there is a fall in the rate of photosynthesis. The initial values are not maintained as can be seen from the individual readings in experiment 39. There is a rapid fall from hour to hour. The time effect sets in very soon at higher temperatures.

A temperature near the optimum most suitable for study of photosynthesis would be about 30° C. At such a temperature the time effect is not marked for a sufficient period, as shown by the constancy of the readings in experiment 37.

TABLE XIV

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF WHEAT, PUSA 4

CO_2 CONCENTRATION, 0.05 PER CENT.
LIGHT INTENSITY, 1875 C.P.

DATE (1933)	EXPERIMENT NO.	TEMPERATURE	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
March 14	32	22.5	1.72
" 15	33	27.0	2.8
" 11	22	30.0	2.82
		°C.	*mg.*

TABLE XV

EFFECT OF TEMPERATURE ON RATE OF CARBON ASSIMILATION OF MATURE LEAVES OF LINSEED, 1150 S

CO_2 CONCENTRATION, 0.05 PER CENT.
LIGHT INTENSITY, 1875 C.P.

DATE (1932)	EXPERIMENT NO.	TEMPERATURE	REAL ASSIMILATION PER 100 SQ. CM. PER HR.
		°C.	*mg.*
February 9	34	22.0	2.72
" 10	35	27.0	3.22
March 18	25	30.0	3.24

With regard to the nature of the reaction, the studies show that VAN'T HOFF's temperature coefficient between 23° and 33° C. would be in the vicinity of 1.4, characteristic of photochemical reactions. As to the exact nature of this reaction and the part it has in the chain of reactions in the photosynthetic process, we are not in a position to say much at present.

Similar responses are found in the case of wheat and linseed leaves (tables XIV, XV).

In these cases the optimum lies near 28° C., a lower temperature than that for sugar cane, probably owing to the comparatively cooler season in which flax grows and the consequent adaptability of the material to temperature.

Discussion

In the preceding pages we have briefly presented a quantitative study of assimilation under various light intensities and carbon dioxide concentration in the light of limiting factors and also of the relative minimum theories. How far these two conceptions hold good under different conditions we shall now discuss.

LIMITATIONS OF LAW OF LIMITING FACTORS

The essence of this law is that only one factor, that which is slowest, governs the rate of the process, which can be accelerated only by increasing the intensity of this factor. For example, if the rate of the reaction is limited by CO_2, only increase in its concentration will increase the photosynthetic rate. Taking a concrete case, we find that under atmospheric concentrations of 0.05 per cent. CO_2 and light intensity of 90 c.p. the rate of assimilation is 1.05 mg. (experiment 2). If we increase the concentration to 0.08 per cent. the rate of assimilation also increases to 1.15 mg. (experiment 3). Under 0.05 per cent. carbon dioxide concentration, therefore, the CO_2 is the limiting factor, for by an increase in its concentration is the rate accelerated. Inquiry as to whether an increase in other factors under the same CO_2 concentration brings about greater assimilatory activity was made, and it was noted that under this limiting condition of CO_2 the rate also could be increased to 1,964 mg. by increasing the light intensity to 1500 c.p. (experiment 9). The readings increase by increasing both the concentration of CO_2 and the light intensity.

Several other experiments were conducted to see whether the peculiarity observed at this light intensity and CO_2 concentration holds good under other conditions. Taking a few examples, the rate of assimilation at 1500 c.p. and 0.12 per cent. CO_2 concentration is 6.1 mg. (experiment 11). When the CO_2 concentration was raised to 0.403 per cent. under the same light intensity the rate of assimilation rose to 6.38 mg. (experiment 12). On the other hand, when under approximately similar concentrations of

0.12 per cent. the light intensity was increased to 1875 c.p. the rate rose to 6.29 mg. (experiment 14). Here also we notice a rise in the activity by changing either the CO_2 concentration or the light intensity to higher units.

These experiments indicate that it is not merely one factor that determines the rate, as BLACKMAN claims, but that the pace of the reaction is determined by at least two factors. Whether by varying the third, namely, temperature, the rate is increased or not we are not in a position definitely to say.

LIMITATIONS OF THEORY OF RELATIVE MINIMUM

As opposed to BLACKMAN'S conception, HARDER (6) holds that of the two factors, light intensity and CO_2 concentration, the one that is present in the minimum governs the pace of the reaction to a greater extent. We have explained the nature of the curves showing the relationship of CO_2 concentration and light intensity on the basis of this theory, but to test how far it holds good under different circumstances a number of experiments were conducted.

Experiment 9 shows that the rate of assimilation under 1500 c.p. and 0.036 per cent. CO_2 is 1.964 mg. An increase in CO_2 concentration to 0.124 per cent., *i.e.,* 3.5 times, increases the photosynthesis to 6.1 mg. (experiment 11), *i.e.,* 3.1 times. In other words, the per unit increase in CO_2 concentration increases the rate by 0.88 times. On the other hand, when the light intensity is increased to 1875 c.p. (1.25 times under more or less similar CO_2 concentrations) the readings are increased to 3.303 mg. (experiment 13), *i.e.,* 1.63 times. In other words, with per unit increase in light intensity the assimilation rate increases 1.3 times. Thus under similar conditions (0.03 per cent. CO_2 concentration) unit increase in CO_2 brings about 0.88 times increase and unit increase in light accelerates 1.3 times, showing that the reaction is more controlled by light even at high light intensities of 1500 c.p. Here the CO_2 concentration is apparently very small while the light is sufficiently high, and therefore the rate, according to the theory of relative minimum, should be governed by CO_2 concentration, an idea contrary to the experimental results obtained.

Several experiments were also carried out to test the applicability of the theory to lower light intensities. Experiment 2 shows the assimilation reading at 90 c.p. and 0.051 per cent. CO_2 concentration. The rate of photosynthesis is 1.05 mg. When the CO_2 concentration is increased to 0.08 per cent., *i.e.,* 1.58 times, the assimilation increases to 1.15 mg. (experiment 3), or 1.09 times. For unit increase in CO_2 concentration, therefore, an increase by 0.69 times is marked in the rate of the reaction. On the other hand, when the light is increased to 1500 c.p., *i.e.,* 16.6 times, the rate under approximately similar concentrations of CO_2 is 1.964 mg. (experiment 9),

an increase of 1.87 times. In other words, for unit light increase there is acceleration by 0.11 times only. Under low light intensity, therefore, the rate is controlled by CO_2 concentration and not by light intensity.

A third aspect remains to be settled, to determine how far the relative minimum theory holds good at high concentrations of CO_2. Several experiments were designed to prove its applicability. Under 375 c.p. and 0.121 per cent. CO_2 concentration the rate of assimilation is 2.391 mg. (experiment 7). This is increased to 2.51 mg., *i.e.*, 1.08 times when the CO_2 concentration is raised to 0.407 per cent., by 3.36 times. On the other hand, when light is raised to 1500 c.p., *i.e.*, by four times under the same concentration, the rate is 6.1 mg., an increase by 2.55 times. For unit increase in CO_2 concentration and light intensity, therefore, there is an increase of 0.32 and 0.637 times respectively. In this case also light is the determining factor.

These observations indicate that the relative minimum theory of Harder does not hold good under all conditions. In the majority of cases light, whether of low or high intensity, controls the rate of the reaction. The main reaction, namely, the relationship of unit CO_2 increase to the rate of assimilation, remains practically the same, the results under low CO_2 concentration and high and low intensities of light being 0.88 and 0.69 times. The increase is practically constant under the different conditions mentioned. Under very high light intensity a slight increase is marked, probably connected with the fact that greater energy is available under the circumstances, which leads to a slight increase in the velocity of the photochemical phase.

We have discussed the limitations of the limiting factor concept as well as of the relative minimum. The evidence relating to interaction of factors favors the view that the rate of photosynthesis is controlled neither by one factor alone (as was held by Blackman) nor by one or a set of two factors present in relative minimum (as claimed by Harder). Most probably each of the factors affecting the rate of photosynthesis has some definite influence on the rate of assimilation, depending upon the intensity of others, the different velocities of the reaction being brought about by changes introduced in the diffusion, photochemical, and chemical stages of the mechanism. Which of these phases is most affected by a particular factor remains to be determined, but since the whole process consists of a series of interlinked reactions, the effect which a change in one factor brings about in a particular phase may induce slight modification in others as well, thus indicating that there cannot be a sharp line of demarcation between the changes produced by one or the other of the factors.

But with all these minor details, the law of limiting factors in its broad sense appears to be of universal applicability and has therefore been a

means of interpreting simply and logically the phenomenon of photosynthesis in its various aspects. To us it seems that any relationship that is traced between the external factors alone, without taking into consideration the internal changes that take place in the mechanism itself, will not hold good under all circumstances. This is well supported by the present observations, and since the actual process is intracellular, the relationship if any has to be sought in the internal concentrations that these factors bring about rather than in the actual external intensities of the factors.

Summary and conclusions

As a preliminary step toward the analysis of growth in terms of assimilation and respiration, it was considered of importance to study the factors affecting these two processes. The present paper is an attempt to elucidate the relationship between the external factors light, temperature, and carbon dioxide and photosynthesis, special attention being paid to the concentrations and intensities in which they interact under ordinary natural conditions in the subtropics.

The investigations have been mainly confined to wheat, linseed, and sugar cane plants and the rate of assimilation was determined by the continuous current methods of BLACKMAN. The following conclusions are the outcome of the present work:

1. Under low light intensities the curves showing the relationship of CO_2 and assimilation are smooth, with no sharp break as observed by BLACKMAN and his coworkers. The stationary phase is either absent or if present extends only to a small range.

2. With higher light intensities also the curves in wheat and linseed are extremely regular, the only difference being that there is a big range of stationary assimilation with increasing concentrations of CO_2, the toxic effect not being so easily marked.

3. The concentration of CO_2 at which the readings are maximum depends upon light intensity. The higher the light intensity the higher is this optimum. These facts are probably correlated with greater availability of energy under high intensities.

4. Under zero percentage of CO_2 the rate of assimilation increases with increasing CO_2, until the respiratory output balances the assimilatory intake of CO_2, namely, the compensation point is reached.

5. Light requirements of different crops vary, probably owing to the nature of the end products formed.

6. Increase in temperature accelerates the rate of photosynthesis, the rise being the result of the accelerating effect of temperature on the one hand and its depressing effect on the other. The readings show a rise until the former is greater than the latter, an optimum being obtained when the two balance each other.

7. The time effect is earlier at higher temperatures.

8. The optimum in the case of wheat and linseed lies at a lower temperature than in the case of sugar cane, probably owing to the colder climate in which these grow and their consequent adaptability toward temperature.

9. It is not one factor that limits the rate of photosynthesis under any set of conditions; the velocity of the reaction is governed by at least two factors.

10. Whether the CO_2 be in high or low concentrations under high intensities of light, the rate is always controlled by light. The theory of relative minimum is limited in application, partially holding good under very low intensities of light and low concentration of CO_2.

11. Any relationship that is traced between the external factors alone, without taking into consideration the internal changes that take place in the process as well as the intensities in which the external factors reach the internal tissues, will not hold good under all circumstances.

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TEMPERATURE AS A PREDETERMINING FACTOR IN THE DEVELOPMENT OF *AVENA SATIVA*[1]

THORA M. PLITT

(WITH THREE FIGURES)

Introduction

It has been shown that various conditions prevailing during the germination of seeds or during the early seedling stages have more or less pronounced effects on the later development of plants. The most comprehensive summary of such investigations is that of KIDD and WEST (**14**). The effects of different temperatures during the germination period on later plant development have been investigated by a number of workers. APPEL and GASSNER (**1**) reported that both summer and winter cereals were injured if their germination took place at unfavorably high temperatures. GASSNER (**10**) brought out the fact that certain types of oats lodged if they were germinated at 25° C. and then transplanted to the field. Lodging occurred even if the 25° temperature prevailed only during the first two days of germination. In either case indications of lodging appeared about five weeks after germination. Under these conditions the oats also failed to head and consequently to yield any grain. The detrimental effects brought about by high temperature were apparently due to disturbances in the very complex chemical reactions in the plant. These findings are confirmed by those of MAXIMOV (**19**). WALSTER (**24**), as a result of an investigation on the formative effects of high and low temperatures on the growth of barley, suggested that the course of development of barley is to a large extent predetermined at a very early stage in growth by the chemical equilibria within the seedling, especially the carbohydrate-nitrogen ratio.

The present investigation was undertaken with the purpose of obtaining some indications as to what differences, if any, are produced in the germinating grains and in the seedlings of *Avena* because of the different temperature conditions prevailing during germination.

Part I

Methods

A. GROWING THE PLANTS

Kherson oats, Nebraska 21 selection, kindly furnished by DR. W. E. LYNESS of the Agricultural Experiment Station of the University of Ne-

[1] Contribution from the Hull Botanical Laboratory, University of Chicago.

braska, were used. The Nebraska 21 oats were selected in 1907 from a field of Kherson oats originating from seed imported from Kherson, Russia, in 1896; this selection has since been grown continuously at the Station.

The grains were first treated with a 0.25 per cent. solution of uspulun for about 20 minutes and then placed on porous porcelain plates in covered glass bowls. Water was placed in the bowls to reach part way up the sides of the plates, and more added later as needed. The bowls were then placed in a refrigerator at 5° or in a Minnesota type germinator at 25° C. according to the condition desired. These two temperatures were chosen because they had been used by GASSNER (10) in his investigation of grains.

At the end of five days the entire grains were dried with absorbent paper, weighed, and placed in sufficient hot 95 per cent. alcohol to make a final extract of 80 per cent. alcohol. About 0.5 gm. of calcium carbonate was added to counteract any acidity in the sample. The whole was heated for about 30 minutes on the steam bath to stop enzymatic action, and set aside for a few days. The extract was then poured off and the grains ground in a mortar before reextraction with 80 per cent. alcohol.

In order to investigate the seedlings, the grains were first treated with 0.25 per cent. uspulun for about 20 minutes and then placed on moist cellucotton in petri dishes. After having been five days in the germinator or the refrigerator, the seedlings were planted in boxes built to fit into glass chambers, about 100 × 90 × 25 cm. These boxes were filled with good garden soil. In these chambers both the temperature and the humidity were controlled. The treatments given the plants are shown in table I.

When the seedlings were three weeks old from the time they were first placed in the refrigerator or the germinator, the tops were cut off at the soil line, separated into two samples, weighed, and treated as previously discussed for the seeds.

The grains in the resting condition were ground in an Enterprise mill, weighed, and extraction carried on as with the seeds. In this and in all

TABLE I

TREATMENT AND GROWING CONDITION OF OATS

LOTS	GERMINATION TEMPERATURE FOR FIRST 5 DAYS	SUBSEQUENT GROWING TEMPERATURE FOR 16 DAYS	PERCENTAGE RELATIVE HUMIDITY DURING THESE 16 DAYS
	°C.	°C.	%
A AA	5	15.0	70
B	25	26.4	70
C	25	26.4	40
D	25	15.5	70
E	5	25.0	70

the above cases, the second extract gave no reaction for reducing sugars, so extraction was deemed to be complete.

B. Chemical analysis

Dry weight.—The percentage dry weight was obtained by heating the plant material in an oven at 103° C. for 30 minutes to stop enzymatic action, then at about 70° C. in a Cenco electric vacuum oven until constant weight was attained.

Soluble carbohydrates.—The 80 per cent. extract was prepared for the soluble carbohydrate determinations by the tentative method XII, 37, (a) of the Methods of Analysis (**2**). Potassium oxalate was used to clear the solution of excess lead acetate as advocated by Loomis (**16**). Inversion was accomplished by the official, first action, method for sucrose XXVII, 22 of the Methods of Analysis (**2**). The resulting reducing sugars were determined by the Kertesz variation of the Bertrand method (**13**). The soluble carbohydrates are calculated as glucose.

Hydrolyzable carbohydrates.—Acid hydrolyzable carbohydrates were determined upon samples taken from the dried and weighed residue left from the alcoholic extract. About 2 gm. of residue were accurately weighed and placed in flasks fitted with reflux condensers. Two hundred cc. of water and 12.5 cc. of concentrated hydrochloric acid were added and the whole boiled gently for three hours. The resulting solution containing the hydrolyzed products was filtered off and the wash water of the residue included with it. This was made up to 500 cc. An aliquot was neutralized with powdered sodium carbonate and the reducing sugars determined by the Kertesz variation of the Bertrand method (**13**). The acid hydrolyzable carbohydrates are calculated as glucose.

Soluble nitrogen.—The soluble nitrogen was determined from the alcoholic extract by the method recommended by Pucher, Leavenworth, and Vickery (**22**) with the variations of T. G. Phillips. These consisted in the use of copper sulphate crystals instead of mercury as a catalytic agent, and the omission of the use of potassium permanganate and of sodium thiosulphate at the completion of the digestion.

Insoluble nitrogen.—Insoluble nitrogen was determined from the residue remaining from the alcoholic extract by means of the official method of Gunning, II, 22 of the Methods of Analysis (**2**).

Total nitrogen is obtained by the addition of soluble and insoluble nitrogen. Likewise total carbohydrates are obtained by the addition of soluble and hydrolyzable carbohydrates.

C. Microchemical examination

The methods used microchemically are as follows:

Pectin.—Ruthenium red (1:10,000): red stain (**8**).

STARCH.—Iodine potassium iodide: indigo. Chloral hydrate added to clear the tissues if the starch grains were small (**8**).

REDUCING SUGARS.—Modified Fehling test of cupric tartrate and 10 per cent. sodium hydroxide, heated at about 40° C. for about two minutes (**8**).

SILICA.—Phenol crystals heated to dissolve them, then clove oil added to prevent recrystallization: pink sheen on silica deposits. The silica method is given in detail by FROHNMEYER (**9**).

PROTEINS AND AMINO ACIDS.—Xanthoproteic reaction: yellow-orange (**8**). Biuret reaction: blue to violet (**8**). Millon's reagent: red (modified by BENSLEY **4**). Ninhydrin: blue. The solution (0.1 gm. ninhydrin to 10 cc. water) is added to the section and left standing for one or two hours. LOEW (**15**) has reported on the use of this reagent.

ALEURONE GRAINS.—Sections are placed in alcohol-ether for 15 minutes to remove fats, then left in borax carmine for about two hours.

HEMICELLULOSE.—Congo red: red stain in walls (**21**). Chlorzinc iodide: blue stain in walls (**21**). Solubility in hot 5 per cent. hydrochloric acid (**8**). Polarizing microscope: walls anisotropic (**21**).

Results

A. GERMINATION

Grains germinated at 5° for five days have a markedly higher percentage of dry weight than grains germinated at 25° C. for five days (table III). Their percentage dry weight based on the original weight of the grains is also higher (table III). There is no question as to the greater imbibition of water by the grains and seedlings at the higher temperature, as is evident from table II. The greater amount of water imbibed is probably due to the fact that the seedlings at 25° were in a more advanced stage of development than the seedlings at 5° C. The seedlings at the higher temperature were in fact 2 to 3 cm. high, whereas those at the lower temperature showed the hypocotyls just breaking through.

TABLE II

INCREASE IN FRESH WEIGHT OF GRAINS WHEN GERMINATED AT 5° AND AT 25° C. FOR 5 DAYS

LOTS	GERMINATION TEMPERATURE	ORIGINAL WEIGHT	WEIGHT AFTER 5 DAYS OF GERMINATION
	°C.	*gm.*	*gm.*
G	5	100	178
H	25	100	248

Germination at the lower temperature resulted in a somewhat higher percentage of hydrolyzable carbohydrates remaining in the grains, as is shown in the calculations based on the original weight of the grains before germination (table III). The higher percentage of hydrolyzable carbohydrates is probably due to the fact that starch hydrolysis proceeds more slowly at low temperatures than at high temperatures. Moreover at the higher temperature the more rapid respiration results in a greater consumption of carbohydrates. The total nitrogen does not, of course, differ materially. Thus the proportion of carbohydrates to nitrogen is somewhat higher at the lower germination temperature. The proportion of soluble to insoluble nitrogen is slightly lower under these conditions.

B. Microchemistry

There are several differences evident in seedlings germinated at the different temperatures. At low temperatures some starch is deposited as very small grains in the parenchymatous tissues of the coleoptile, the coleorhiza, the scutellum, the cortex of the root, and the root cap. At the higher temperature, however, starch is not deposited in the seedling, or if so, only a little which tends to disappear soon. In the resting grain there is no starch in the embryo.

Another difference is in the amount of pectin produced. At low temperature there is pectin all through the coleoptile and the coleorhiza. It is especially abundant in the epithelium of the scutellum. There is some pectin present in the epiblast and in the root cap. At the higher temperature pectin is present in similar amounts in the coleoptile, the coleorhiza, the epithelium of the scutellum, and the epiblast. But there was a great deal more pectin in the root cap and in addition there was considerable pectin in the epidermis of the root. The seedlings which at this higher temperature had attained a height of about 2 cm. exhibited a great deal of pectin in the cell walls of the coleoptile. Pectin was especially prominent in all of the cell walls of the bundles of the coleoptile except the lignified xylem elements. Reducing sugars could not be detected in the embryo of the resting grain. In the grains germinating at 5° C. reducing sugars could not be detected until the third day; even then there were only traces present which could not be localized satisfactorily. By the fifth day there was not much more in evidence. On the other hand, in grains germinating at 25° C. traces of sugar could be detected after one day. On the second day it was found present in the parenchyma of the root and coleoptile.

The majority of the seedlings germinated for five days at 25° C. were 2 to 3 cm. high, as stated previously. These seedlings were high in reducing sugars, particularly in the cortical parenchyma and the epidermis of the stem; some appeared also in the primary leaves. This confirms the

TABLE III

ANALYSIS OF GRAINS IN THE RESTING CONDITION AND AFTER GERMINATION FOR 5 DAYS AT 5° AND AT 25° C. CALCULATIONS MADE ON BASIS OF DRY WEIGHT (UPPER PART OF TABLE), ON BASIS OF FRESH WEIGHT (MIDDLE PART OF TABLE), AND ON BASIS OF ORIGINAL WEIGHT BEFORE GERMINATION (LOWER PART OF TABLE)

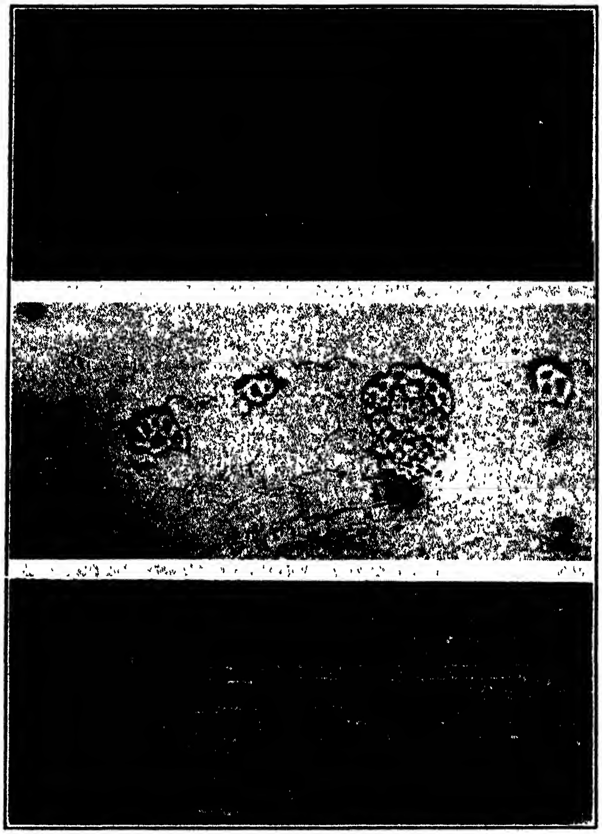
	Condition	Dry weight	Soluble carbohydrates	Hydrolyzable carbohydrates	Total carbohydrates	Hydrolyzable CH / Soluble CH	Soluble nitrogen	Insoluble nitrogen	Total nitrogen	Insoluble N / Soluble N	Carbohydrates / Nitrogen
		%	%	%	%		%	%	%		
F	Resting	91.9	1.528	60.92	62.45	$\frac{100}{2.5}$	0.071	1.683	1.754	$\frac{100}{4.2}$	36.77
G	5° C. 5 days	53.39	0.205	54.67	54.88	$\frac{100}{0.4}$	0.101	1.801	1.902	$\frac{100}{5.6}$	28.85
H	25° C. 5 days	37.86	2.428	52.53	54.96	$\frac{100}{4.6}$	0.120	1.901	2.021	$\frac{100}{7.9}$	27.19
F	Resting	91.9	1.404	56.00	57.40	$\frac{100}{2.5}$	0.065	1.547	1.612	$\frac{100}{4.2}$	36.43
G	5° C. 5 days	53.39	0.110	29.19	29.30	$\frac{100}{0.4}$	0.054	0.962	1.016	$\frac{100}{5.6}$	28.73
H	25° C. 5 days	37.86	0.926	19.89	20.82	$\frac{100}{4.6}$	0.057	0.720	0.777	$\frac{100}{7.9}$	26.79
G	5° C. 5 days	95.04	0.195	51.96	52.16	$\frac{100}{0.4}$	0.096	1.712	1.808	$\frac{100}{5.6}$	28.84
H	25° C. 5 days	90.86	2.207	47.73	49.94	$\frac{100}{4.6}$	0.137	1.727	1.864	$\frac{100}{7.9}$	26.79

macrochemical findings of a considerably higher percentage of soluble sugars when germinated at 25° than when germinated at 5° C. (table III).

The protein and amino acid tests, xanthoproteic, biuret, Millon, and ninhydrin, indicated a more rapid mobilization of these substances when germination took place at 25° than at 5° C. In the resting grain all these stains were positive in the embryo and the aleurone layer. The layer of large cells immediately below the aleurone layer was stained particularly with Millon's reagent. In the aleurone layer there are present many aleurone grains (borax carmine method); in these aleurone grains there are no inclusions. During germination at the lower temperature the growing point, the primary leaves, and the root gradually take deeper stains. The ninhydrin test shows the most change. Whereas in the resting grain only a faint blue rim of color is obtained about the edge of the cover glass, as germination proceeds the color becomes deeper and localized in the regions just mentioned; moreover this reagent seems to stain the vascular tissues particularly. At the higher germination temperature after one day the primary leaves and the root stand out. After two days the biuret reaction is purple in the growing point and the primary leaves, but bluish in the coleoptile and the root (especially the stele), possibly indicating differences in the protein units present. After three days in the germinator ninhydrin yields a deep blue color in the root tip, the stele of the root, the primary leaves, particularly in the vascular bundles, and in the bundles of the coleoptile. These reactions continue through the germination period.

It was noted that in the resting grain the walls of practically all parenchyma cells are irregularly thickened, particularly at the corners. These thickenings are stained blue by chlorzinc iodide and red by Congo red. They are soluble when the sections were heated to the boiling point in 5 per cent. hydrochloric acid. Examination of the sections with the polarizing microscope before treating with acid showed these thickenings to be anisotropic; but after the acid treatment this quality was lost. As growth proceeds these thickenings decrease slightly in the original regions but appear to become more prominent in the parenchyma of the leaves. This may be an instance of hemicellulose cell wall thickenings, possibly functioning as a reserve food.

Some 6-weeks old plants were examined for the distribution of silica. These plants had been germinated at 5° or at 25° C. for five days and had then been transplanted to a plot of soil in the greenhouse. Of the plants that were germinated at 5° C. individuals were examined which stood erect. The silica was deposited mainly in the walls of the short epidermal cells of the stem (fig. 1). There was also some deposition in the cell walls of the stomata. The irregular occurrence of circular dark spots in the walls of the long epidermal cells of the stem also represent silica deposited in the



FIGS. 1–3. Fig. 1, epidermis of stem of 6-weeks old oat plants showing silicon deposit in cell walls of short cells particularly, some in stomatal cell walls, and traces in circular areas in long cells. Fig. 2, cross section of a leaf sheath of a 6-weeks old oat plant with silicon deposited in certain epidermal cells over the bundles. Fig. 3, epidermis of a leaf sheath of a 6-weeks old oat plant with silicon deposited in separate cells arranged in rows.

cell walls as a fine network. Figure 2 shows the cross section of a leaf sheath, and figure 3 the longitudinal view. It will be noted that the silica is deposited in epidermal cells over the bundles, but that these cells as seen in top view are separated from one another by cells whose walls do not contain silica. A few of those plants which were germinated at 25° C. had lodged. These were examined for silica, but no differences were found between them and the erect plants.

C. After three weeks

The two series in the controlled chambers were run three weeks apart, but in immediate succession: May 5 to 26 and May 24 to June 15, 1931.

TABLE IV

ANALYSIS OF PLANTS 3 WEEKS OLD GROWN AT 15.5° C. (FIRST AND THIRD PARTS OF TABLE) AND AT 25°–26.4° C. (SECOND AND FOURTH PARTS OF TABLE). UPPER HALF OF TABLE CALCULATED ON DRY WEIGHT BASIS; LOWER HALF ON FRESH WEIGHT BASIS

	Growing temperature	Relative humidity	Germination temperature	Dates	Dry weight	Soluble carbohydrates	Hydrolyzable carbohydrates	Total carbohydrates	Hydrolyzable CH / Soluble CH	Soluble N	Insoluble N	Total N	Insoluble N / Soluble N	Carbohydrates / Nitrogen	Height
	° C.	%	° C.		%	%	%	%		%	%	%			cm.
A	15.5	70	5	5/5–5/26	10.55	2.65	9.09	11.74	$\frac{100}{29.1}$	0.846	5.591	6.437	$\frac{100}{1.5}$	1.83	11.4
AA	15.5	70	5	5/24–6/15	11.36	6.00	12.10	18.10	$\frac{100}{49.9}$	0.917	5.450	6.367	$\frac{100}{16.8}$	2.84	11.6
D	15.5	70	25	5/24–6/15	12.12	14.60	18.57	33.17	$\frac{100}{78.6}$	1.464	8.682	10.146	$\frac{100}{16.9}$	3.28	11.8
B	26.4	70	25	5/5–5/26	12.36	8.33	12.14	20.47	$\frac{100}{68.6}$	0.760	4.800	5.560	$\frac{100}{15.8}$	3.68	19.6
E	25	70	5	5/24–6/15	11.91	5.61	12.37	17.99	$\frac{100}{45.4}$	1.115	4.363	5.478	$\frac{100}{25.6}$	3.78	18
A	15.5	70	5	5/5–5/26	10.55	0.279	0.959	1.238	$\frac{100}{29.1}$	0.090	0.611	0.701	$\frac{100}{15.1}$	1.78	11.4
AA	15.5	70	5	5/24–6/15	11.36	0.676	1.375	2.051	$\frac{100}{49.9}$	0.105	0.619	0.724	$\frac{100}{16.8}$	2.66	11.6
D	15.5	70	25	5/24–6/15	12.12	1.769	2.250	4.019	$\frac{100}{78.6}$	0.198	1.052	1.250	$\frac{100}{16.9}$	3.23	11.8
B	26.4	70	25	5/5–5/26	12.36	1.030	1.501	2.531	$\frac{100}{68.6}$	0.094	0.594	0.688	$\frac{100}{15.8}$	3.68	19.6
E	25	70	5	5/24–6/15	11.91	0.668	1.475	2.143	$\frac{100}{45.3}$	0.133	0.520	0.655	$\frac{100}{25.6}$	3.28	18.2

The increased day length in the second period increased all of the carbohydrate fractions, and did not cause any appreciable change in the nitrogen fractions (table IV, A and AA). Notwithstanding this increase in carbohydrates the two series are comparable, for the results show the same trend. Thus the proportion of carbohydrates to nitrogen is less in both A and AA, germinated at low temperature, than in B or D, germinated at the higher temperature.

At the end of three weeks the seedlings grown at about 25° (table IV, B and E) were the taller regardless of the other conditions applied in these experiments. Seedlings germinated at 25° (table IV, B and D as compared with AA and E) exhibit a higher percentage of dry matter than those germinated at 5° C., also higher percentages of soluble carbohydrates and generally a higher percentage of total carbohydrates. There is a decidedly higher proportion of soluble to hydrolyzable carbohydrates: 68.6:100 and 78.6:100 (B, D) as compared with 49.9:100 and 45.4:100 (AA, E).

Those plants which were germinated at 25° C. and grown at about the same temperature (table V, B) were higher in all the carbohydrate fractions and slightly lower in all the nitrogen fractions than those germinated at 5° and grown at 15° C. (table V, A). The total carbohydrates are 8 per cent. higher and total nitrogen is about 0.9 per cent. lower; hence their proportion of carbohydrates to nitrogen is decidedly higher. The proportion of soluble to hydrolyzable carbohydrates is also far higher than with the other treatment; 68.6:100 (B) at about 25° C. compared with 29.1:100 at low germination and growth temperatures (A).

At this age (three weeks) the proportions of soluble to insoluble nitrogen did not seem to be correlated with any specific factor. For instance, the proportion of insoluble to soluble nitrogen is 100:25.6 when germination took place at 5° and growth at 25° C. (table V, E); but another lot (AA) germinated at 5° and grown at 15.5° C. has a proportion of 100:16.8, and yet another lot (B) germinated at 25° and grown at 25° C. has a proportion of 100:15.8.

One lot of oats was germinated at 25° C. and grown at the same temperature but at a relative humidity of 40 per cent. (table VI, C) instead of 70 per cent. (B). These plants did not produce so rank a growth as the plants grown at a humidity of 70 per cent., as may be seen from the measurements of their heights (table VI); and their percentage of dry matter was slightly higher. The proportion of soluble carbohydrates to hydrolyzable carbohydrates is slightly less. Since the percentage of total carbohydrates is less but the percentage of nitrogen about the same, the ratio of carbohydrates to nitrogen is slightly less when the humidity is decreased.

Discussion

The greater imbibition of water and the greater growth of the seedlings at the end of the 5-day germination period with a temperature of 25° as compared with the behavior of seedlings grown for the same length of time at 5° C. is in agreement with the general findings as summed up by BARTON-WRIGHT (3). He states that not only does the temperature affect the rate of entry of water into the seed as well as the growth rate of the radicle, but it also affects the resistance of the seed coat to the extrusion of the radicle. *Phaseolus vulgaris* shows evidence of germination at 9° C., and the growth rate increases with rising temperatures to 36° and ceases at 46° C. Minimum, optimum, and maximum germination temperatures for rye are 1°, 25°, and 36° C. respectively. The optimum points are somewhat dependent on the time factor.

The results obtained concerning the amount of carbohydrates present in the low temperature seedlings are in agreement with the findings of DICKSON, ECKERSON, and LINK (7) that 10-day old wheat seedlings grown at low soil temperatures are practically high carbohydrate plants.

It is well known that plants respire more rapidly at higher temperatures than at lower ones. This fact is reflected in the percentage of dry weights obtained at the end of the first five days of germination at different temperatures. DAY (6), in working with barley, measured the carbon dioxide output for ten days at three different temperatures. He found that the average output per hour was greater at 60° F. than at 38°–43°, and still greater at 70°. Another feature was that the respiration increases to a maximum and then decreases again. At the lowest temperature given, this maximum occurs on the fourth and fifth days. But at the highest temperature it is shifted to the third day.

It seems likely that the greater amount of soluble sugar present in oat grains germinated for five days at 25° C. is associated with the fact that no starch is deposited in the embryo under those circumstances, whereas starch is deposited at the lower temperatures usually considered more favorable for the growth and development of this plant. In this respect the behavior of oats is similar to that of barley as reported by BROWN and MORRIS (5).

Increase in pectin materials at high germination temperature in oats parallels the results obtained in wheat (7).

Lower germination temperatures are generally recommended as being more favorable for growth and development than higher ones. The different rates of hydrolysis of storage materials and of synthesis of new materials at higher temperatures as compared with lower ones may conceivably affect the later development of plants, directly or indirectly. Thus at the lower temperature the starch in the endosperm is hydrolyzed more rapidly than the protein; also there is less vegetative growth than at the higher

TABLE V

Analysis of plants 3 weeks old germinated at 5° (upper portion of table) and at 25° C. (lower portion of table); calculated on dry weight basis

	Germination temperature	Growing temperature	Relative humidity	Dates	Dry weight	Soluble carbohydrates	Hydrolyzable carbohydrates	Total carbohydrates	Hydrolyzable CH / Soluble CH	Soluble nitrogen	Insoluble nitrogen	Total nitrogen	Insoluble N / Soluble N	Carbohydrates / Nitrogen	Height
	°C.	°C.	%		%	%	%	%		%	%	%			cm.
A	5	15.5	70	5/5 5/26	10.55	2.65	9.09	11.74	100/29.1	0.846	5.591	6.437	100/15.1	1.83	11.4
AA	5	15.5	70	5/24 6/15	11.36	6.00	12.10	18.10	100/49.9	0.917	5.450	6.367	100/16.8	2.84	11.6
E	5	25.0	70	5/24 6/15	11.91	5.61	12.37	17.99	100/45.4	1.115	4.363	5.478	100/25.6	3.78	18.2
B	25	26.4	70	5/5 5/26	12.36	8.33	12.14	20.47	100/68.6	0.760	4.800	5.560	100/15.8	3.68	19.6
D	25	15.5	70	5/24 6/15	12.12	14.60	18.57	33.17	100/78.6	1.464	8.682	10.146	100/16.9	3.28	11.8

TABLE VI

ANALYSIS OF PLANTS 3 WEEKS OLD, GERMINATED AT 25°, GROWN AT 26.4° C., BUT AT DIFFERENT RELATIVE HUMIDITIES: 40 AND 70 PER CENT. CALCULATED ON BASIS OF DRY WEIGHTS (UPPER PART OF TABLE) AND ON FRESH WEIGHT BASIS (LOWER PART OF TABLE)

	Relative humidity	Growing temperature	Germination temperature	Dates	Dry weight	Soluble carbohydrates	Hydrolyzable carbohydrates	Total carbohydrates	$\frac{\text{Hydrolyzable CH}}{\text{Soluble CH}}$	Soluble nitrogen	Insoluble nitrogen	Total nitrogen	$\frac{\text{Insoluble N}}{\text{Soluble N}}$	$\frac{\text{Carbohydrates}}{\text{Nitrogen}}$	Height
	%	°C.	%		%	%	%	%		%	%	%			cm.
B	70	26.4	25	5/5 5/26	12.36	8.33	12.14	20.47	$\frac{100}{68.6}$	0.760	4.800	5.560	$\frac{100}{15.8}$	3.68	19.6
C	40	26.4	25	5/5 5/26	13.74	5.73	10.67	16.40	$\frac{100}{53.7}$	0.840	4.629	5.469	$\frac{100}{18.1}$	3.00	14.1
B	70	26.4	25	5/5 5/26	12.36	1.030	1.501	2.531	$\frac{100}{68.6}$	0.094	0.594	0.688	$\frac{100}{15.8}$	3.68	19.6
C	40	26.4	25	5/5 5/26	13.74	0.787	1.466	2.253	$\frac{100}{53.7}$	0.118	0.633	0.751	$\frac{100}{18.1}$	3.01	14.1

temperature (**7**). In the present investigation both the macrochemical and the microchemical data seem to indicate that the rates of carbohydrate and protein hydrolyses and utilization are definitely affected by an increase in germination temperature. ZALESKI (**26**) reports that as the protein in *Lupinus augustifolius* is broken down, much asparagin appears. The amount of asparagin obtained increases with rise in temperature. The temperature coefficient follows the VAN 'T HOFF rule, $Q_{10} = 2.5$. The indications are that the slower rate of hydrolysis of protein at lower temperatures in oats makes for conditions of growth more favorable to the complete development of the plant.

The most outstanding characteristic of the 3-weeks old plants which had been germinated at 25° C., regardless of the subsequent growing conditions here considered, was the marked increase of total carbohydrates in the tops of the plants over those germinated at 5° C. These high temperature plants had already reached the stage at which photosynthetic activity might begin, at the time the others were just breaking through the seed coats. GREGORY (**12**) found in *Cucumis sativus* that the growth rate of leaf surface increases with the temperature, and that it is dependent on the area of the leaf surface already present. But on investigating the relative rate of increase (rate of increase per unit of leaf surface already present) he found it to be independent of the temperature. He states that the differences in the final leaf area between plants grown at different temperatures must therefore be related to the time elapsing from germination before the photochemical process can begin, *i.e.*, the time elapsing before expansion of the foliage leaves begins. This is a matter of growth and differentiation in the apex and has what he calls a normal temperature coefficient, $Q_{10} = 2.5$. Thus the delay in the development of the first leaf is determined by the temperature at which the developmental processes occur. He believed that a nitrogenous leaf-forming substance is involved. Thus any later differences in leaf surface are caused by the speed with which the plant attained its first leaf. The present experiment seems to point to the conclusion that, since the seedlings at the higher temperature were further developed when transplanted from the 25° germinator to the chambers than those transplanted from the 5° C. refrigerator, they had the advantage of an earlier start in photosynthetic activity which expressed itself in a higher percentage of carbohydrates in the tops of the plants at the age of three weeks.

Analyses of older plants, such as those of clover by TOTTINGHAM (**23**), indicate a higher percentage of polysaccharides in plants grown at 16.9° to 23.3° C. than in those grown at 23.2° to 28.2° C. It may be a complicating factor that in this experiment he collected the plants grown at the higher temperature two weeks sooner than the others, in order that all might have

attained the same size for analysis. Yet he again obtained in buckwheat grown at 16.0 to 19° C. a percentage of polysaccharides higher than when grown at 20.5° to 25° C., with all of them harvested at the end of ten weeks. He attributes the decrease mainly to the impossibility of polysaccharide storage at higher temperatures because of greater consumption of sugars with increased respiration. On the other hand it is probable that different optima exist for different stages of development of a plant. GASSNER (**10**) recommends for Uruguay oats a low temperature germination followed by a rapid rise in temperature in order to achieve maturity as early as possible.

Furthermore McLEAN (**20**), in investigating effects of climatic conditions, using soy beans, came to the conclusion that temperature was the limiting condition for growth during the first two weeks in practically all cases. During the second two weeks of growth, however, with exactly the same environmental conditions, the moisture relation (rainfall-evaporation ratio) appears to have been the limiting condition, especially if the temperature was high. This must be due to a difference in the internal conditions of the plants at the different developmental stages. Growth during the early period consisted largely in stem elongation, which must have been accomplished at the expense of material stored in the seed. The rate of development of the plants was therefore probably dependent on the rate of hydrolysis of storage materials and of translocation from the cotyledons to the growing points, and hence dependent on the temperature. On the other hand, during the second two weeks while leaf expansion occurs, greater transpiration will increase the water requirement. So the moisture relation would be a greater factor than before.

APPEL and GASSNER (**1**) report that various cereals, such as wheat, barley, and oats, raised entirely in the warm greenhouse germinated rapidly and continued to grow rapidly. They had already attained a height of 15 cm. when those in the cool greenhouse germinated. After three weeks, however, the rate of growth of those at the high temperature decreased so that finally they were overtaken by those at low temperature. WALSTER (**24**) noted a similar phenomenon. Barley plants in the warm house, which were several inches high before the plants in the cool house had come up, during the first two weeks maintained a more rapid growth. But at the end of a month the plants in the cool house had outstripped those in the warm house in their growth rate. At the end of six weeks all of the plants in the cool house had outgrown those in the warm house.

Analyses of plants at later stages than those made in this investigation may well yield interesting results.

The findings concerning the highly localized deposition of silica agree with those made by WYSSLING (**25**) in his survey of the distribution of silica in plants. Since the deposition of silica does not result in a continu-

ous structure, and since silica is only a very small part of the mechanical structure of the stem and leaf sheaths, it seems unlikely that it should be a factor in determining an erect or a recumbent habit of the plant. Rather, as set forth by WYSSLING, it seems to be an excretion of a nonessential element absorbed in different quantities under differing conditions.

Part II

Other varieties of oats were tested for possible responses to different germination temperatures. Through the courtesy of the Agricultural Experiment Station at the University of Tennessee four varieties were obtained, Hatchett, Lee, Turf B, the Fulghum 699–2011. From the State College of Agriculture at the University of Georgia were obtained three other varieties, Fulghum, Norton, and Appler. Each of these varieties was subjected to the following four germination conditions: (*a*) 2° for 5 days; (*b*) 25° for 5 days; (*c*) 2° for 2 days, then 25° for 3 days; (*d*) 25° for 2 days, then 2° for 3 days. Treatment was begun on May 27, 1932. At the close of the 5-day period the germinating grains or seedlings were planted in a plot of soil in the greenhouse. On September 19 the experiment was terminated.

Observations were made on dates of heading and possible evidences of lodging. In this series there appeared to be no clear correlation between the temperatures prevailing during germination and dates of heading (these dates were late in the season for oats). Dates of heading differed within a single variety, but no general trend was observable when comparing the different varieties. As for evidences of lodging, there was no bending of the culms in the lower nodes or internodes. In a few cases the culms were leaning over or even broken, but this appeared to be the result of the method of watering. At times the temperature of the greenhouse rose considerably, even reaching 124° F. (51.1° C.). It seems obvious that these varieties of oats do not give a lodging response to high temperatures applied either early or late in the life of the plants.

Part III

In order to approximate more closely the experimental material reported by GASSNER (**10**), some South American varieties of oats were tested. These were secured from Dr. ALBERTO BOERGER, Director of the Agricultural Experiment Station at Montevideo through the courtesy of Mr. JOSE RICHLING, Consul General of Uruguay in New York. These oats are classified as follows: 1095a belongs botanically to the species *Avena byzantina;* BID belongs to the species *A. satia,* originating from a hybrid of two forms of this species; 64s, *A. capa,* represents a type native of Uruguay and belongs to the species *A. sativa.* GASSNER reports using "*Avena* del pais"

or native Uruguay oats. The seeds falling to the ground in the field make pastures which are good for several years. BOERGER states that although none of these samples was harvested in fallow land, it would be perfectly feasible to obtain them from such land owing to the fact that fallen seeds of any kind soon become transformed into what are called "avena gaucha" (wild oats) offering valuable pasture for winter and fall seasons.

The investigation of the response of these oats to germination temperatures was undertaken in the laboratories of the Botany Department at Columbia University through the courtesy of Professor SAM F. TRELEASE. The oats were treated as previously, namely, germinated at 25° and 5° C. for five days and then planted in the greenhouse. Treatment of the grains was begun on March 25, 1933, and the plants were harvested on June 8.

No lodging was observed in any of these plants.

One lot of the oats headed, namely, the 64s oats which were germinated at low temperature (5° C.). All were bearing grain when harvested. A parallel series, but with germination taking place at 25° C., bore no grain whatsoever. Of the BID oats (5° C. germination) only one culm headed; of the 1095a oats none headed. The varieties are evidently quite distinct from one another, for BOERGER reports the increased yield of the 64s oats. There are also differences in the vegetative portions of the plants: 1095a produces thin culms and fine leaves, BID thicker culms and broader leaves, and 64s the sturdiest plants.

Such a difference in yield was reported by GASSNER (**10**) when working in Uruguay: Uruguay oats kept for the first ten days at 6°–9° C. headed after two months, likewise those kept for ten days at 6°–9° then for two days at 25° C., and also those kept for the first five days at 6°–9° and then for two days at 25° C. But oats germinated at a constant temperature of 25° C. failed to head by the end of eight months; even those which were exposed to a temperature of 25° only the first two days and then at 6°–9° C. for eight days failed to head.

GASSNER (**11**) further differentiates between summer and winter cereals. According to him the latter require a certain period of cold at some time in the life cycle in order eventually to go over into the reproductive phase. The summer cereals, on the other hand, will go over into the reproductive phase even without undergoing such a cold period, although they also appear to be influenced by such an exposure. MAXIMOV (**19**), in testing *Avena byzantina,* found that as the germination temperature was decreased from 26° to 0° C. the vegetative period was shortened from 80 days to 31 days, and simultaneously the yield was increased. The principle of shortening the length of the vegetative stage of grains seems to be applied on a large scale in Russia under the name of Jarovization. The methods em-

ployed and a consideration of the factors involved are presented by LYSSENKO (**17, 18**).

It was thought that higher germination temperatures than 25° C. might cause greater disturbances in the growth of the oat plants. From June 20 to July 24 a number of plantings were made in the greenhouse at the University of Chicago; these were observed until November 24, 1933. The oats were: Kherson oats Nebraska 21 selection, and the oats 1095a, 64s, and BID from Uruguay. The grains were first germinated for five days at 5°, 10°, 25°, 30°, 32.5°, 35°, 37.5°, and 40° C., then planted in soil plots.

The last two temperatures, 37.5° and 40° C., were evidently fatal, for none of the grains placed in these germinators ever germinated. At 35° C. there was a low percentage germination and subsequent growth was limited. The other stands were fairly good; in no case, however, was any lodging observed.

Only one variety headed, namely, the Kherson oats (Nebraska 21 selection). This is very possibly due to the late date of seeding, for during the spring certain of the 64s oats did head. But of these Nebraska 21 oats only those germinated at 10° and 5° C. headed. These were placed in the refrigerators on June 20 and 26 respectively, and they began to head on August 22 and 29. In this respect these oats were similar to the 64s oats in the preceding series. In no case was any lodging observed. All through the growing period many of the plants were stooling, some of the plants having forty or more culms of all sizes at the end of the experiment. The factor operating to produce this unusual result was not determined.

Summary

1. Oat grains germinated at 25° C. for five days have a markedly lower percentage of dry weight than grains germinated at 5° C. when calculated either on the basis of fresh weight or on the basis of the original weight before germination. These differences are directly related to the rate of growth of the seedlings at the two temperatures, and the amount of carbon and hydrogen consumed in respiration. A somewhat lower percentage of starch remains in the grains when germinated at the higher temperature, and more water is imbibed by the grains than at the lower temperature. The proportion of soluble to insoluble nitrogen is slightly higher at the higher germination temperature.

2. Microchemically it was found that whereas there is no starch in the embryo of oat grains in the resting condition, starch is deposited in the embryo during germination at 5° C.; but little or none is deposited in the embryos germinated at 25° C. At the higher germination temperature there are more pectin and reducing sugars in the seedling than at the lower temperatures.

3. Protein reactions (microchemical) became more intense as germination proceeded, gradually in the case of the lower temperature, much more rapidly at the higher temperature.

4. Cell wall thickenings were found in the parenchyma of the embryo and the early seedling stages of the oats, giving reactions indicating the presence of hemicellulose.

5. Seedlings grown at about 25° C. were the tallest in the series regardless of the germination temperature; they also had the lowest percentages of nitrogen.

6. Seedlings which grew from grains germinated at 25° C. regardless of the later conditions applied in these series exhibited a higher percentage of dry matter than those germinated at 5° C., also higher percentages of carbohydrates, and the nitrogen percentages were lower. There is a higher proportion of soluble to insoluble acid hydrolyzable carbohydrates.

7. Plants germinated and grown at about 25° C. were higher in carbohydrates and slightly lower in nitrogen; also the proportion of soluble to insoluble acid hydrolyzable carbohydrates is far higher than in plants germinated at 5° and grown at 15° C.

8. The proportions of soluble to insoluble nitrogen varied.

9. Oats germinated and grown at 25° C. but at a relative humidity of 40 per cent. as compared with those at 70 per cent. made less growth; their percentage of dry matter was slightly higher, and the percentages of carbohydrates were lower.

10. The greater amount of carbohydrates in plants germinated at 25° as compared with that in plants germinated at 5° C. is believed to be caused by the fact that the seedling development is much further advanced at the time of transplanting, so that photosynthetic activity begins several days earlier.

11. Silica in 6-weeks old plants was found to be deposited in the same manner in both erect and recumbent plants. It is highly localized in certain cells and these cells form no consecutive structure. Hence silica is believed to be a negligible factor in the supporting mechanism of the plant.

12. High temperatures during the very early phases of germination of oats had much less effect on lodging of oats than the work of GASSNER indicates. No temperature treatment during early germination was found that would consistently cause lodging to develop, either immediately or in later life.

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ABSORPTION OF SULPHUR DIOXIDE BY ALFALFA AND ITS RELATION TO LEAF INJURY[1]

MOYER D. THOMAS AND GEO. R. HILL, JR.

(WITH FIVE FIGURES)

Introduction

A knowledge of the amount of sulphur dioxide which must be absorbed by vegetation in order to produce a definite amount of leaf destruction, rather than the amount to which vegetation may be exposed, is fundamental to an understanding of the smoke problem. It is particularly important to know the relations existing between the rate of absorption and the total quantity of absorption of the gas and the extent of leaf destruction, since it will be shown that sulphur dioxide absorbed in the leaves of alfalfa is rapidly converted into a much less toxic form. Moreover, the rate of absorption in a given plant system varies widely under different environmental conditions. The most important consideration, therefore, is not the concentration of the sulphur dioxide in the air alone, but rather the concentration, the duration of the fumigation, and the rate at which the gas is absorbed by the plant, taken together. Accordingly an exposure which might cause severe injury under one set of conditions might be entirely harmless under another set. In the absence of data showing the rate of absorption it is impossible to say whether the different effects which are produced by a given set of exposure conditions are due to different quantities of gas absorbed or to different degrees of susceptibility.

Earlier work in this field has been concerned primarily with the effect of duration and intensity of exposure to sulphur dioxide in producing injury on vegetation. A study of the absorption of the gas by chemical analysis of the leaves has usually been incidental and apparently carried out to show that the sulphur content of the tissue actually increased owing to the fumigation treatment. The analytical data in the literature, therefore, do not show definitely the amount of absorbed gas which was associated with a definite amount of leaf destruction. STOKLASA states (**4**, p. 156) that 0.175 per cent. SO_2 in lupines, 0.379 per cent. in corn, and intermediate amounts in other crops (expressed as percentage of the dry plant tissue) have been observed to exert a harmful influence. These data seem to represent the

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order of magnitude of the effect at least for short fumigations. In many cases, however, material for the analyses was not taken immediately before and immediately after the fumigation treatment, in which cases the analytical data are of very doubtful value for indicating the absorption effect. It has been shown in an earlier paper from this laboratory (8) that satisfactory absorption data can be obtained either by analyses of the leaves for total sulphur or by continuous analysis (6, 7, 8) of the SO_2-air mixture before and after contact with the vegetation. In the former method great care is necessary in taking the samples of vegetation. The latter method offers the advantage that it eliminates sampling errors and also shows the progress of the absorption. Its principal disadvantage lies in the necessity of applying a correction because of absorption of the gas on the walls of the fumigation chamber.

In this paper the amounts of absorbed sulphur dioxide which cause different amounts of leaf destruction in alfalfa are considered. Also the relations existing between the duration and intensity of exposure are analyzed with reference to rate of absorption of the gas by the plant and the attendant leaf destruction. Most of the experimental data have been obtained by the method of continuous air analysis.

Experimental method and results

During the seasons from 1927 to 1931 a considerable number of fumigation experiments were conducted with the idea of studying the rate of absorption of sulphur dioxide by alfalfa and the relation of the quantity of absorbed gas to the leaf destruction. The work of 1927 was of a preliminary nature, but in 1928 and subsequent years quantitative absorption data were obtained. The fumigation apparatus and technique (1) and also the analytical methods (6, 7, 8) have already been described. Field plots 5 feet square were covered with a gas-tight celluloid-covered cabinet through which a controlled mixture of SO_2 and air was passed. The concentrations of sulphur dioxide and the air volumes at both the intake and outlet of the cabinet were determined. The light intensity, temperature, and relative humidity in the cabinet were also measured. A careful estimate was made of the percentage of leaf area destroyed by fumigation on each plant, as described in the preceding paper (1). Many representative stems were taken from the field at intervals of 5 to 7 days throughout the growth of the crop, from which the growth curve, and ratio of leaves to stems, as well as leaf area, were found. From this information and the harvest weight of the crop, the weight and area of the leaves at the time of fumigation could be closely approximated. Further, the amount of gas absorbed when the cabinet was placed on bare soil was studied. The absorption by the soil was found to

be appreciable, but when the ground was covered with celluloid only a small amount of absorption could be attributed to the cabinet, unless the relative humidity was more than 80–90 per cent. In the fumigation work, all exposed soil under the plants was covered with celluloid and the absorption data were corrected for the amount of absorption by the cabinet under similar conditions of fumigation.

The experiments for 1928 to 1930, inclusive, are plotted in figure 1, in which the ordinate is the percentage of leaf area destroyed, and the abscissa the amount of sulphur dioxide absorbed, calculated as parts per million of the dry weight of the leaves. The duration of all the fumigations in charts A and B was between 60 and 100 minutes. The data are segregated into four groups with different types of points, according as the plots had full sunlight or were shaded, and according as the relative humidity was more or less than 80 per cent.

In spite of the fact that the individual points of each group in figure 1 are scattered over an appreciable area of the chart, they clearly tend to follow a straight line course, thus showing that the percentage of leaf destruction is in direct proportion to the amount of sulphur dioxide absorbed. If any relationship other than linearity exists between leaf destruction and absorption of sulphur dioxide, it is not evident in figure 1. The equations of the best fit straight lines, found by the method of least squares through the different types of points in figure 1, are as follows:

Curve A: Summarizing fumigations in which the plants were exposed to full sunlight and the relative humidity was less than 80 per cent.:

$$x = 822 + 35.6y \qquad (1)$$

in which y is percentage of leaf destruction, and x is SO_2 absorbed, calculated as parts per million of dry leaf tissue. The equation states that it requires the absorption of 822 p.p.m. SO_2 to cause any leaf destruction at all, and the absorption of 4382 p.p.m. to bleach the leaves completely, under the conditions which obtained in these experiments. The coefficient of correlation, r, of this curve is 0.792, and the standard deviation of an individual absorption, S_x, is 527 p.p.m. The number of fumigations represented, n, is 131, and their average duration 1.35 hours.

Curve B: Summarizing experiments in which the plants were shaded and the relative humidity was less than 80 per cent.:

$$x = 1003 + 38.5y \qquad (2)$$

$n = 67$

$r = 0.795$

$S_x = 538$ p.p.m.

Average duration of fumigation = 1.43 hours.

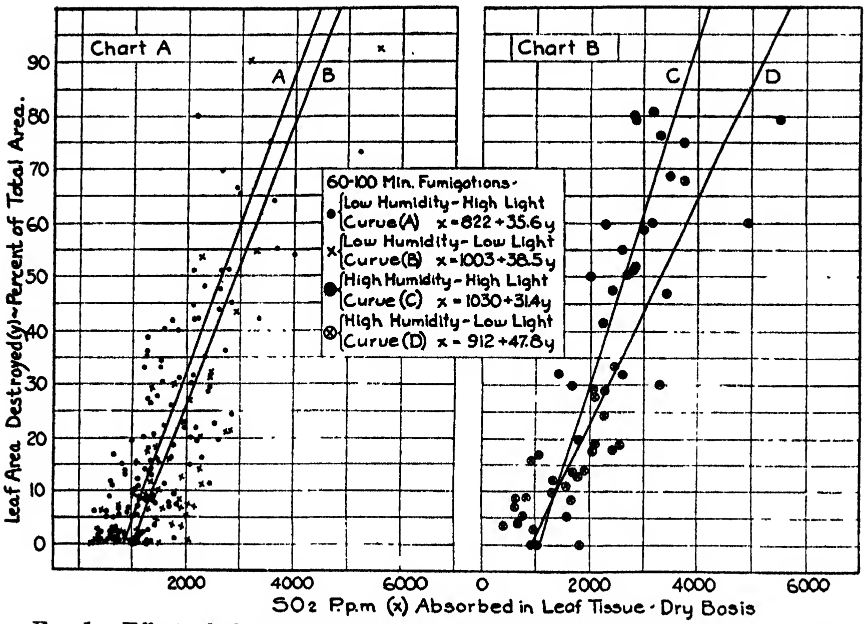

FIG. 1. Effect of absorption of different amounts of sulphur dioxide, calculated as p.p.m. of dry leaf tissue, upon destruction of the leaves of alfalfa under different conditions of light, humidity, and duration of exposure, as indicated in legend. Equations found by method of least squares. 1928–1930 data.

Curve C: Summarizing fumigations in which the plants were exposed to full sunlight and the relative humidity was more than 80 per cent.:

$$x = 1030 + 31.4y \quad (3)$$

$n = 29$

$r = 0.817$

$S_x = 581$ p.p.m.

Average duration of fumigation = 1.19 hours.

Curve D: Summarizing fumigations in which the plants were shaded and the relative humidity was more than 80 per cent.:

$$x = 912 + 47.8y \quad (4)$$

$n = 25$

$r = 0.864$

$S_x = 536$ p.p.m.

Average duration of fumigation = 1.23 hours.

These equations, as plotted in figure 1, show that there is a tendency for the SO_2 absorbed to be slightly less effective in destroying the leaf tissue under conditions of low light, but the differences are not great. This is further confirmed by equations (7) and (8) below. No significance can be

attached to the fact that curves C and D cross each other near the zero ordinate, because of the relatively small number of experiments in each group and their relatively large standard deviations. At very high humidities the absorption by the walls of the cabinet is high, and accordingly the absorption values obtained under these conditions are inherently more uncertain than are the low humidity results. It is likely that when the humidity approaches saturation, particularly in the shade, appreciable amounts of the gas are absorbed on the exterior of the leaves and stems. At low relative humidity, on the other hand, external surface absorption is of minor importance and has probably been allowed for adequately in the "cabinet correction." This is indicated by the fact that in many fumigations, when the plants were inactive, the absorption, by both the plants and the cabinet, exceeded the cabinet correction only slightly.

When all the data in figure 1 are placed in one group, the equation and statistics of the best fit straight line are as follows (curve F, fig. 2):

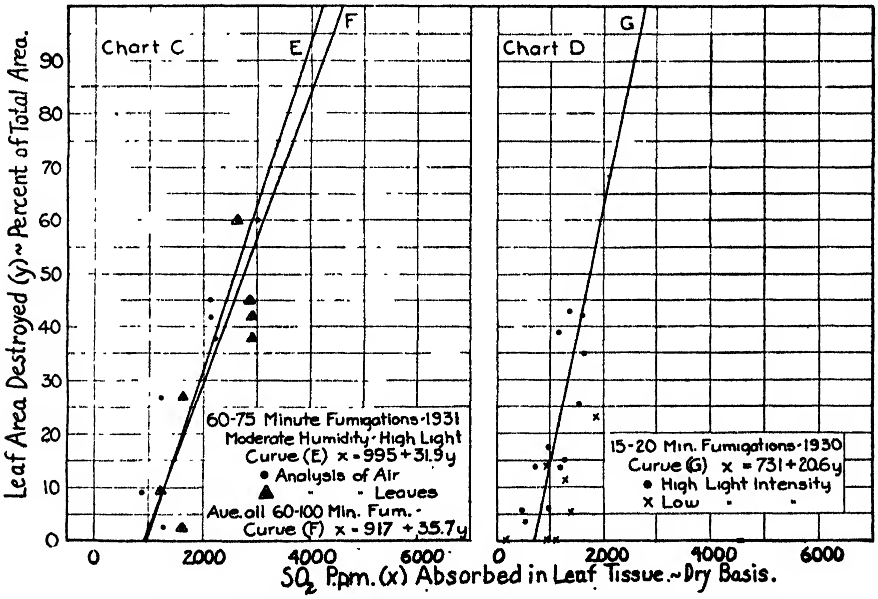

FIG. 2. Effect of absorption of different amounts of sulphur dioxide, calculated as p.p.m. of dry leaf tissue, in different time periods, in causing the destruction of the leaves of alfalfa.

$$x = 917 + 35.7y \qquad (5)$$

$n = 252$

$r = 0.809$

$S_x = 557$ p.p.m.

Average duration of fumigation = 1.34 hours.

This result is only slightly different from that obtained by combining equations (1) and (2):

$$x = 896 + 35.6y \quad \text{(6)}$$
$$n = 198$$
$$r = 0.782$$
$$S_x = 541 \text{ p.p.m.}$$

Average duration of fumigation = 1.38 hours.

The coefficients of correlation associated with equations (1) to (6) are all about 0.8, which may be interpreted as indicating a definitely significant degree of correlation. On the other hand, the standard deviations of the absorption values from the curves in the individual experiments are about 550 p.p.m., and therefore an appreciable range of leaf destruction can be expected with the same rate of absorption under different conditions. Of course the dispersion of the data is partly due to the fact that these experiments include treatments of duration ranging from 60 to 100 minutes, which would necessarily imply different rates of absorption, as discussed later. Finally it must be emphasized that the experimental difficulties involved in the accurate measurement of the absorption are great. In general the experiments were conducted so that the concentration of SO_2 in the air was reduced only about 5 to 15 per cent. in passing through the fumigation chamber. An error of 1 per cent. in the measurement of the difference in concentration between intake and outlet would therefore cause an error of 7 to 20 per cent. in the absorption value.

Equation 1 has been fairly well confirmed by a few carefully executed experiments conducted in 1931, which have been reported in detail elsewhere (8). The absorption of sulphur dioxide was determined both by gas analysis and by analysis for total sulphur of samples of leaves taken immediately before and after the fumigation. These data are plotted in chart C, figure 2. The sulphur analyses show somewhat larger absorption values than do the gas analyses, probably owing to the fact that in taking the leaf samples there was a tendency to pick an unduly large proportion of the upper leaves, which had absorbed the gas more freely than the less active lower leaves. The equation and statistics of the best fit straight line through the absorption values, determined by gas analysis alone, are as follows:

$$x = 713 + 33.9y \quad \text{(1a)}$$
$$n = 7$$
$$r = 0.932$$
$$S_x = 244 \text{ p.p.m.}$$

Average duration of fumigation = 1.1 hours.

The equation of the line determined by the leaf analyses alone is:

$$x = 1273 + 30.0y \quad \text{(1b)}$$
$$r = 0.825$$

$$S_x = 389 \text{ p.p.m.}$$

and by the average of the gas and leaf analyses:

$$x = 995 + 31.9y \quad \text{(1c)}$$
$$r = 0.900$$
$$S_x = 289 \text{ p.p.m.}$$

These equations represent experimental conditions comparable with those which obtained in equation (1). Equation (1c) is plotted as curve E in figure 2. As already mentioned, great care was exercised in carrying out these particular experiments, so that in spite of their meager number they may be regarded as offering definite confirmation of equation (1). The differences are within the observed experimental errors and variations.

Three series of short fumigations of about 15–20 minutes' duration have also been carried out. Because of time required to displace the large volume of air in the cabinet, the concentration of SO_2 in these experiments rose rapidly to a maximum over a period of 4–6 minutes, and after remaining approximately constant at this level for about 14–18 minutes, fell to zero over a period of 4–6 minutes, unless the humidity was very high, in which case the cabinet continued to lose SO_2 for about 20 minutes after the supply had been stopped. The average duration of the peak concentration in these experiments was 17 minutes, but appreciable amounts of SO_2 were present for 21 minutes on the average, and detectable amounts were registered for 28 minutes. Owing to these rapidly changing conditions in the cabinet, accurate absorption data could be obtained only if two SO_2 autometers were sampling continuously from the intake and outlet of the system. In practice each machine sampled from both intake and outlet, but the operation was timed so that one drew its sample from the intake while the other was sampling from the outlet. The machines were stopped momentarily in the middle of the experiment to reverse the source of sampling in order to eliminate machine differences.

The absorption values in these experiments are plotted in figure 2. The two series at high light intensity conform to the equation:

$$x = 667 + 20.2y \quad \text{(7)}$$
$$n = 14$$
$$r = 0.779$$
$$S_x = 234 \text{ p.p.m.}$$

Average duration of fumigation = 0.35 hours.

The data of all three series conform to equation (8), which is shifted somewhat to the right of equation (7). This shift lends support to the statement previously made that the absorbed gas is slightly less effective in destroying leaf tissue in the shade than in direct sunlight.

$$x = 731 + 20.6y \quad \text{(8)}$$
$$n = 20$$

$r = 0.664$
$S_x = 315$ p.p.m.
Average duration of fumigation = .35 hours.

Equations (7) and (8) indicate considerably smaller absorptions for a certain amount of leaf destruction than equations (1) to (6). This means that if a definite amount of leaf destruction is produced in different time periods, the longer fumigations require lower concentrations of gas but greater total absorptions than the shorter fumigations. Further confirmation of this fact is evident in the case of long-continued fumigations at very low concentrations. For example, the analytical data of an experiment have been presented (8) in which alfalfa leaves absorbed, during a period of 19 days, nearly 12,000 p.p.m. sulphur, equivalent to nearly 24,000 p.p.m. sulphur dioxide calculated on the dry weight of the leaf tissue. The sulphur was largely retained in the leaves as sulphate. In this experiment only 1.0 per cent. acute markings were produced, owing to accidental peaks of concentration, although chlorotic markings had developed at the end of the experiment to the extent of 14 per cent. of the leaf area. It is suggested that the chlorotic markings were caused by this large accumulation of sulphate, which finally may have interfered with cell functions, including that of chlorophyll formation.

General time-concentration-absorption equations for alfalfa

It seems evident from the above discussion that the amount of injury caused by a given quantity of sulphur dioxide varies with the rate of absorption of the gas; *i.e.*, a given sufficient amount of gas, absorbed in a short time period, will cause more leaf destruction than if the absorption period is more protracted. It also seems evident that some gas can be absorbed without causing any leaf destruction, the quantity thus disposed of being greater the more slowly it is absorbed. Chemical evidence in a following paper suggests that the absorbed sulphur dioxide is largely and rapidly oxidized and neutralized in the leaf. It is evident that the sulphate radical which is a normal and considerable constituent of the tissue is practically non-toxic, and if the rate of absorption does not exceed a certain threshold value, the sulphur dioxide can all be oxidized and neutralized without causing injury to the cells. The more the rate of absorption exceeds this value, the less completely will it be oxidized and neutralized and the more injury will it cause. As suggested above, in the case of sufficiently long-continued fumigations at low concentrations, it is possible that the accumulation of sulphate finally reaches a value which may interfere with the cell functions, possibly either by exceeding the buffer capacity or by producing a salt effect on the cell components. Alfalfa at Salt Lake seems to have a tolerance for sulphur dioxide absorbed thus slowly some thirty times greater than when

absorption is rapid. The fumigations on which the equations and conclusions in this paper are based were of such short duration that large accumulations of sulphate were not built up, and therefore no account is taken of this factor. The limitations of the absorption equations thus imposed will be discussed later. The data represent primarily the toxic action of sulphur dioxide itself, with such partial conversion into non-toxic forms as occurs in these short periods of fumigation.

The absorption leaf-destruction relations developed here are of limited scope, since they are concerned with fumigations of two durations only. More general relations are needed connecting leaf destruction with such variables as duration of fumigation, concentration of sulphur dioxide in the air, rate and quantity of absorption by the vegetation, and rate of conversion of the absorbed SO_2 to a less toxic form. A simple mathematical treatment is outlined below, by which some of these general relations may be found.

Consideration of equation (1) indicates that the absorption value x is made up of three principal variables: time, t; concentration of SO_2 in the air, C; and an absorption factor, A, which is a measure of the activity of the plant in absorbing the gas and varies with the external environment as well as internal conditions. The equations can therefore be written in the more general form:

$$x = x_0 + Ky \qquad (9)$$

or

$$x = tAC = tAC_0 + Ky \qquad (10)$$

where

$$x_0 = tAC_0$$

represents absorption conditions attending incipient leaf destruction ($y = 0$), and K is a constant. For definite values of y, the term Ky is constant and can be rewritten in the form, kA (k is another constant), so that

$$tAC = tAC_0 + kA \qquad (11)$$

For each value of y, the absorption is thus a linear function of t. Since equation (10) has been evaluated for fumigations of two durations, as represented by equations (1) and (7) for example, it is possible to solve equation (11) for its constants assuming definite values of y. A family of curves can thus be calculated showing the amounts of absorption in different time periods which can cause any specific degree of leaf destruction. To illustrate, assuming incipient leaf destruction, the absorption is 822 p.p.m. in 1.35 hours (equation 1) and 667 p.p.m. in 0.35 hours (equation 7). Substituting these values and solving for the constants, equation (11) becomes:

$$tAC = 610 + 155t \qquad (12)$$

Equation (12) states that a minimum absorption of 610 p.p.m. SO_2 (based on the dry leaf tissue) would cause incipient leaf destruction if the gas could be taken up instantaneously ($t = 0$). If the rate of absorption is less rapid, additional sulphur dioxide, proportional in amount to the time required for the whole quantity to be absorbed, is necessary to produce this effect.

If equations (5) and (8), which also represent similar external conditions, are employed, somewhat larger constants are obtained but the relations are not greatly changed:

$$tAC = 664 + 188t \quad \text{(12a)}$$

By similar operations, employing equations (1) and (7), 50 per cent. leaf destruction yields the equation:

$$tAC = 1350 + 925t \quad \text{(13)}$$

and 100 per cent. leaf destruction:

$$tAC = 2090 + 1700t \quad \text{(14)}$$

Equations (12), (13), and (14) are plotted in figure 3. The intercept on the Y axis, or the first constant in the equations, represents the amount of absorption that would be required to produce the specific effect if the gas could all be added instantaneously; that is, 2090 p.p.m. for 100 per cent., 1350 p.p.m. for 50 per cent., and 610 for incipient leaf destruction. These values would be increased to 3790, 2275, and 765 p.p.m. respectively, if the absorption period were one hour. These curves are not dependent on the value of A in equation (11), since this factor and C may vary reciprocally without changing the value of the absorption in any given case. The curves illustrate in striking manner the inactivation of part of the absorbed gas as the rate of absorption falls off, or conversely they show that a given amount of absorbed gas is more active the more quickly it is taken up by the plant.

If sufficiently large values of the time (t) are inserted in equations (12) to (14), quantities of absorbed SO_2 are indicated which are physically impossible. It is clear, therefore, that the equations must be used subject to limitations imposed by the biological system. As already pointed out, the equations were developed from data obtained in fumigations of comparatively short duration, and their extrapolation to fumigations of longer duration must be made with caution. This matter will be treated more fully in a subsequent paper. It may be pointed out that the equations will remain valid for larger values of the time the smaller the amounts of leaf destruction under consideration. Assuming the suggested interpretation of the reactions,—that part (or all) of the sulphur dioxide is converted into the non-toxic sulphate form and if sufficient gas is absorbed the remainder exerts an immediate direct harmful action,—the accumulated sulphate will probably remain non-toxic as long as: (1) it can be neutralized as rapidly

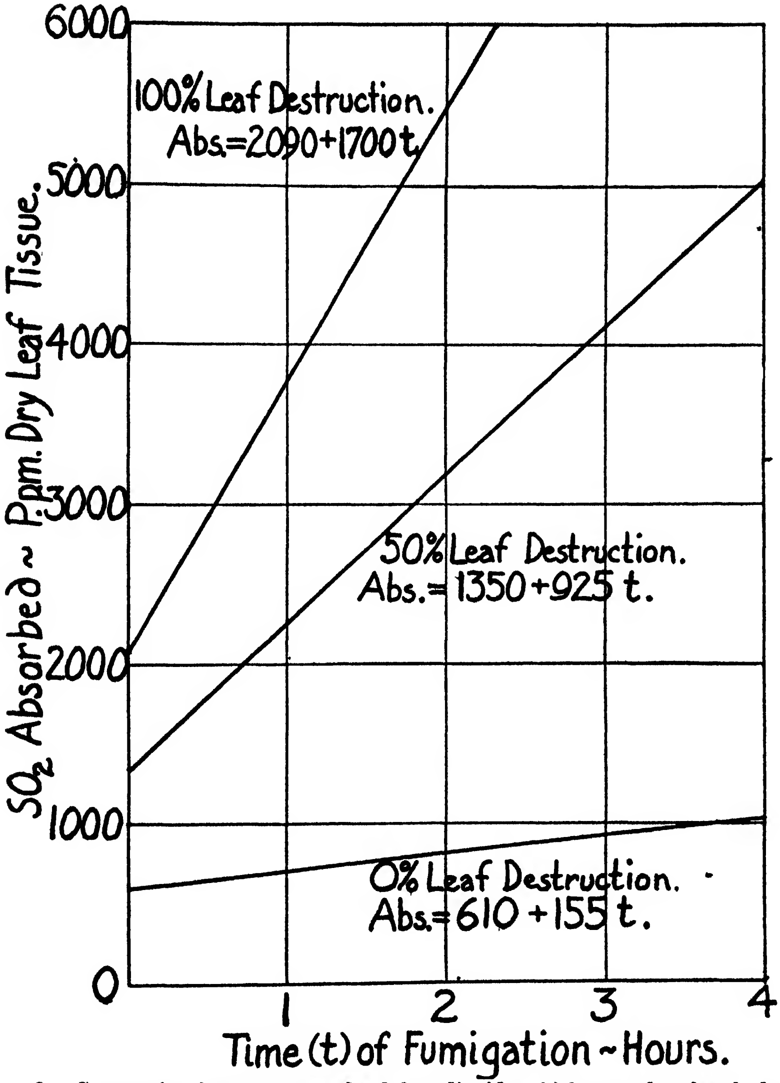

FIG. 3. Curves showing amounts of sulphur dioxide which must be absorbed in different time periods in order to produce three different stages of leaf destruction in alfalfa.

as it is formed, although it cannot be said at this time how much free sulphuric acid the cell can tolerate; and (2) its total concentration does not interfere with the cell metabolism. The first condition will depend on the quantity of bases in the system and will doubtless be different in plants grown on different types of soil. No information is available at present as to whether the second condition is subject to appreciable variation in dif-

ferent localities. It has been observed that in the presence of as much as 1.5 to 2.0 per cent. of sulphur as sulphate in the dry leaf tissue of alfalfa grown at this laboratory, the plants are normal, as indicated by yield, composition, and appearance.

By eliminating A from equation (11) we have:

$$tC = tC_0 + k \qquad (15)$$

which is identical in form with an exposure equation developed by O'GARA (3, 5) and which will be discussed later. Equation (15) indicates that there is a reciprocal relation between time of exposure and concentration of the SO_2 in the air in producing a definite amount of leaf destruction. This relation will hold, however, only if the rate of absorption A is constant in the whole series of exposures embraced by equation (15). If A is not constant in the whole series, equation (15) does not follow from equation (11).

The factor A is a measure of the absorptive rate of unit (dry) weight of the leaves and is mathematically independent of the duration and intensity of the fumigation. Physiological and environmental influences will cause the value of A to vary over a wide range and the absorbed SO_2 may cause it to change during a short fumigation treatment. Examination of the 1928 to 1931 absorption data reveals that the value of A ranged from a minimum of about 30 to a maximum of 650 in the different daylight experiments. The dimensions of A were so chosen that when the time was expressed in hours and the concentration of SO_2 in the air in parts per million by volume, the absorption was given as parts per million of the dry leaf tissue. The same maximum value of A (650) was observed in both the 15–20-minute and the 60–100-minute fumigations, but A was generally smaller in the longer treatments under comparable conditions owing to the fact that it frequently decreased during the course of the treatment.

If it is desired to know the time-concentration exposure relations which will produce a given amount of leaf destruction with a definite rate of absorption, it is necessary only to divide the appropriate equation of the family to which equations (12) to (14) belong by the chosen absorption rate factor A. For example, if the equation representing incipient marking under conditions of maximum sensitivity is sought, equation (12) should be divided by:

$$A = 650$$

$$tC = 0.94 + 0.24t \qquad (16)$$

A corresponding equation for 50 per cent. leaf destruction is:

$$tC = 2.1 + 1.4t \qquad (17)$$

and for 100 per cent. leaf destruction:

$$tC = 3.2 + 2.6t \qquad (18)$$

These curves are plotted in figure 4, and represent minimum values of the

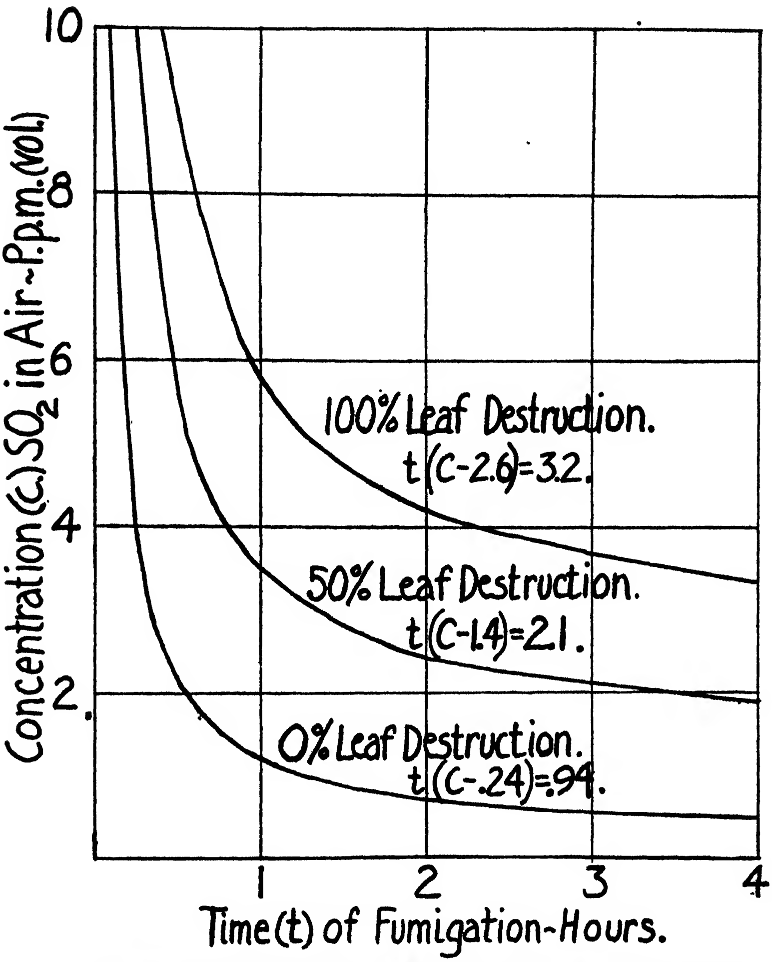

FIG. 4. Curves showing concentrations of sulphur dioxide which will produce three different stages of alfalfa leaf destruction in different time periods under conditions of maximum absorption ($A = 650$).

sulphur dioxide concentration in the air which will produce a trace, 50, or 100 per cent. leaf destruction respectively in alfalfa, under conditions of most rapid absorption. Actual fumigations will generally require higher concentrations than those indicated, because an absorption rate of $A = 650$ is not often attained, particularly in the longer fumigations which usually show a decreasing value of A as the fumigation proceeds.

As already mentioned, O'GARA developed an exposure equation like equation (15). He observed the concentrations of the gas which produced incipient markings in alfalfa with different times of fumigation. Then

using an empirical equation connecting gas concentrations with relative humidity, he adjusted his concentration values to correspond with 100 per cent. relative humidity, which was assumed to represent a condition of maximum sensitivity of the alfalfa. These adjusted values are plotted in figure 5 against the time of fumigation. The curve O'GARA drew through the *minimum* values of concentration conforms to the equation:

$$tC = 0.92 + 0.33t \qquad (19)$$

This curve is clearly a reasonable interpretation of the data, although slightly different constants might have been chosen. The fact that it is not materially different from equation (16) lends support to the mathematical treatment of the absorption data presented.

O'GARA's interpretation of equation (19) was as follows:

"With all environmental factors remaining the same, the active part of the gas necessary to produce a certain effect upon the plant cell, multiplied by the time through which it acts is constant" (**3**, **5**).

This is more clearly shown if the equation is rearranged:

$$t(C - 0.33) = 0.92 \qquad (19a)$$

According to O'GARA, the constant 0.33 represents a threshold concentration to which the plants can be subjected indefinitely without producing markings under conditions of maximum sensitivity, and the "active" part of the gas in any fumigation can be found by subtracting 0.33 p.p.m. from the actual concentration. This interpretation of the exposure factors is analogous to that just given for the absorption values. The similarity of the concepts is based on the implicit condition that throughout a given series of fumigations a definite fraction of the gas is absorbed. In that case absorption is directly proportional to exposure concentration. Ordinarily this assumption is not justified and a large part of the fumigation data will not conform closely to these simple relations, as indicated by many of the individual points in figure 5. Of course both the exposure and the absorption data are subject to variations such as are represented by the dispersion of the points in figures 1 and 2.

Further confirmation of the preceding mathematical treatment and interpretations may be found in the barley fumigation data of the Selby Smelter Commission (**2**). Two examples which are typical of the results may be quoted: Severe lesions were produced by 8–10 p.p.m. SO_2 in one-half hour; 5 p.p.m. in one hour; or by 1 p.p.m. for 43 hours intermittently on 6 days. Slight leaf destruction was produced by 6, 12, 18, or 27–36 fumigations, each of 10 minutes' duration, when the gas concentration was 5, 3, 2, or 1 p.p.m. respectively. While no mathematical treatment of the Selby data has been attempted, it is clear that equations of the type of (15) could be worked out for at least several of the series of fumigation treat-

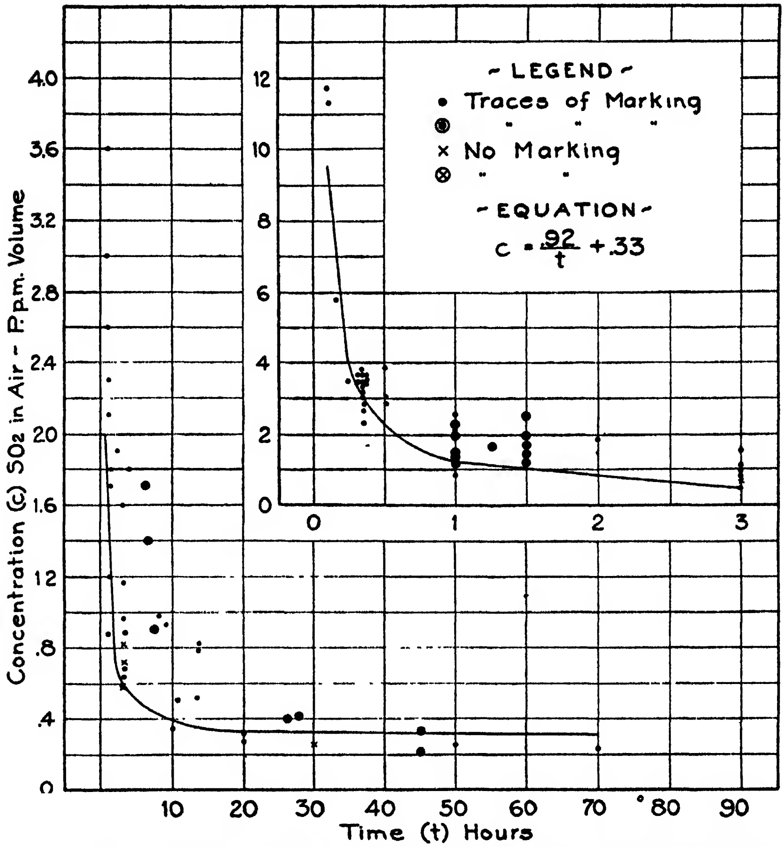

FIG. 5. O'GARA's data showing concentrations of sulphur dioxide, adjusted to 100 per cent. relative humidity by an empirical equation involving the relative humidity of the air, which cause no more than traces of markings on alfalfa in different times of exposure. Circled points represent data obtained by the writers.

ments. Again, such regularities as might be found would depend on a duplication of conditions in a series so that the absorption factor would be approximately constant.

Toxic dosage of SO_2 for the mesophyll cells

It seems desirable to attempt to estimate, on the basis of the preceding data, the amount of SO_2 which is lethal to the absorbing cells of the leaf. It has been shown that the absorption in an infinitesimally short time period of 610 p.p.m. SO_2, calculated on the dry leaf tissue, produces incipient marking, and that the absorption of 2090 p.p.m. under similar conditions causes complete leaf destruction. The different cells of a leaf do not absorb the gas with equal rapidity, because of unequal areas exposed to the gas.

Accordingly the first cells to show injury have undoubtedly absorbed considerably more than the average amount per cell found for the whole leaf. On the other hand, absorption does not stop when injury appears, and therefore the absorption which attends complete leaf destruction probably represents much more than the lethal dosage for a considerable portion of the leaf. The most probable value of the lethal dosage for the injured section lies between these extremes, and is represented by the average, 1350 p.p.m., dry basis, or about 270 p.p.m. SO_2 based on the green weight of the leaf. It should be noted that the absorption occurs principally in the mesophyll, since this tissue is particularly adapted by its very large and continuously moist exposed area for the rapid absorption of gases. On the other hand, the water proofed epidermis and the compact vascular tissue, with its comparatively small area exposed to the air, probably play an insignificant part in this gaseous absorption. If these latter tissues represent 50 per cent. of the leaf substance, which seems to approximate the correct value, the toxic dosage for the remaining mesophyll would be twice the average for the whole leaf. The figures, therefore, of

2700 p.p.m. SO_2 in dry mesophyll cells

and

540 p.p.m. SO_2 in green mesophyll cells

are suggested as probably representing the order of magnitude of the lethal quantity of this gas.

Summary

1. The amount of sulphur dioxide absorbed by the leaf tissue of alfalfa has been measured in a considerable number of fumigation treatments.

2. As a first approximation, the extent of leaf destruction is a linear function of the amount of SO_2 absorbed in a given time period.

3. An appreciable amount of gas can be absorbed without causing any leaf destruction.

4. The amount of leaf destruction attending a given amount of absorption increases as the rate of absorption is increased.

5. For a definite amount of leaf destruction, for relatively high exposure, the amount of absorption is a linear function of the time required for the absorption.

6. An absorption factor, A, which depends on the activity of the leaf in taking up the gas, is discussed. It is shown that if this factor is constant in a series of experiments, the absorption equation can readily be transformed into time-concentration exposure equations. In this way the fumigation data of O'Gara and the absorption data in this paper lead to practically identical equations for the exposure conditions which produce incipient marking in alfalfa under conditions of maximum sensitivity. Fur-

ther, it is possible to calculate from the absorption equations the exposure condition for any specified amount of leaf destruction or value of A.

7. The evidence indicates that when the rate of absorption exceeds a certain threshold value, a specified amount of injury appears in a time which is proportional to the difference between the actual rate of absorption and the threshold rate. The cells of the leaf can dispose of a certain amount of the gas by oxidation and neutralization, but injury occurs if the gas is supplied so rapidly that these processes cannot be maintained, thus permitting a sufficient accumulation of unoxidized or unneutralized suphur dioxide.

8. Assuming that the lethal dosage of sulphur dioxide is added to the leaf instantaneously, and that it is all absorbed by the mesophyll cells, which represent about one half of the leaf substance, it is suggested that the limit of tolerance to the gas of these cells is approximately:

2700 p.p.m. in dry tissue

and

540 p.p.m. in fresh tissue.

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INFLUENCE OF DIFFERENT QUANTITIES OF MOISTURE IN A HEAVY SOIL ON RATE OF GROWTH OF PEARS[1]

M. R. Lewis, R. A. Work, and W. W. Aldrich

(WITH FIVE FIGURES)

Introduction

In 1930 studies were initiated in the Medford area to determine so far as possible the best methods of irrigation of the heavy soils of the area for the production of large yields of high quality pears. One of the primary problems encountered was that of the effect of different soil moisture conditions at different times during the growing season on the yield of fruit. During the first two years complete records of soil moisture conditions and of the size and yield of fruit at harvest were secured. Study of these results brought out the urgent need for more detailed data on the effect of moisture conditions on the rate of growth of fruit from day to day. This information was secured during the 1932 growing season. An analysis of the rate of growth of fruit throughout the season as compared with the corresponding soil moisture conditions is presented in this report. The results obtained from this analysis are applied to the soil moisture conditions of the two earlier years with a satisfactory check on the size of fruit at harvest.

During each of the three years, studies were conducted on two commercial orchards designated as the Fitch and Klamath orchards. During 1932 additional work was done at the Medford Experiment Station. Both soils and trees in these orchards are extremely variable, but in each case the plots were selected for uniformity of soil type and tree growth. Mature bearing trees were selected. The treatment of the different plots in each orchard up to the time of the beginning of these experiments was uniform so far as is known. The trees on the Fitch orchard are Bartletts while on both the Klamath and Medford Experiment Station orchards the variety is Anjou, all on French roots.

[1] The data reported in this paper have been secured in the course of studies on the irrigation of pears carried on cooperatively by the U. S. Department of Agriculture and the Oregon Agricultural Experiment Station near Medford, Oregon. All of the work in 1930 and 1931 and the irrigation and soil moisture studies in 1932 were conducted by R. A. Work under the direct supervision of M. R. Lewis. Detailed studies of fruit growth and tree response were conducted in 1932 by W. W. Aldrich. The method of interpreting the data as reported in the present paper has been developed by the senior writer.

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The climate is semi-arid with a comparatively long growing season and almost continuous sunshine during the summer months.

Irrigation was by the furrow method, six to eight furrows being used in each middle. Irrigation water was available on demand except after early July in the 1931 season. The Fitch and Klamath orchards were cultivated once after each irrigation. The Medford Experiment Station orchard was cultivated once in early summer when the winter cover crop was disced in, and again once late in the summer.

The soils in these orchards are very heavy, locally known as "sticky," and are typical of a large part of the pear orchard land in the Medford area. The soil of the Fitch orchard is mapped as Meyer silty clay loam and that of the other two orchards as Meyer clay adobe. Analyses of typical samples of these soils are reported by the Bureau of Soils (**7**). A sample of the Meyer clay adobe from an adjoining field of the Medford Experiment Station had the following mechanical analysis (**6**): total sand 36.4 per cent., silt 25.3 per cent., and colloidal clay 38.1 per cent.

The soil is from 3 to 6 feet deep and rests on a somewhat disintegrated shale. The water table is below the surface of the rock at all times except occasionally just after a heavy irrigation when it may rise above the rock surface for a few days.

Plan of experiments

In each orchard four plots were laid out with border rows and with from 5 to 16 trees in inner rows which were considered satisfactorily uniform within each orchard for use in the study of the effect of soil moisture conditions on the growth and yield of fruit. The moisture content of the soil in the different plots was permitted to drop to various percentages of the available capacity of the soil before being replenished by irrigation.

Samples for soil moisture determinations were taken just before and as soon after each irrigation as it was possible to do so without too much difficulty with the sticky mud. Samples were also taken at approximately 2-week intervals when periods between irrigations permitted. Samples were taken in increments of 1 foot in depth, starting below the mulch and extending to the bed rock. Representative samples of the soil of each plot secured in the Fitch and Klamath orchards from five holes and in the Medford Experiment Station orchard from twenty holes were used for all determinations. Methods used in determining soil moisture constants in these studies are described elsewhere (**9**).

The location of the feeding roots of the trees in these orchards has been investigated both by actual count and weight of roots recovered from trenches and by study of the rate of extraction of moisture from the soil. The data (**2**) indicate that almost all of the roots are in the upper 4 feet,

and that of those about 89 per cent. are in the upper 3 feet. The rate of loss of soil moisture from depths below 3 feet is very slow and as a result the variation in soil moisture below that depth is comparatively small. For these reasons the moisture content of the upper 3 feet of the soil has been used as representing the moisture available to the trees. The moisture content is presented in terms of the percentage of the available capacity present at any time. In other words, the moisture content of the plots shown on the curves as 0 per cent. is the wilting point and the content shown as 100 per cent. is the field capacity.

The rate of growth of fruit during 1932 in the Fitch and Klamath orchards was determined by measuring the circumferences of 45 pears on each plot at intervals of from 3 to 7 days. These measurements were made from May 17 to August 11, about one week before the first picking of the Bartlett pears on the Fitch orchard, and to September 7, two days before picking of the Anjous on the Klamath orchard. At the Medford Experiment Station orchard 90 fruits per plot were measured at 3-day intervals from May 23 to September 8.

The actual volume of individual fruits of these varieties approximates that of a sphere having the same circumference. The volumes of spheres corresponding to measured circumferences rather than actual volumes of fruits have therefore been used throughout this study.

The fruit from each plot was run through mechanical graders in the packing houses separately and the number of pounds of each size of fruit was determined.

Results

Table I gives the average field capacity, wilting point, and available capacity of the soil in the three orchards. Results for individual plots are reported elsewhere (**9**).

Study of the effect of different soil moisture conditions on the yield of fruit (**8**) indicated that in these plots the trees suffered from lack of soil moisture before the soil in the upper 3 feet had reached the wilting point. ALDRICH and WORK (**1**) have shown that differences in the moisture content of the upper 3 feet of the soil below 50 per cent. of the available capacity but well above the wilting point resulted in differences in fruit size and branch growth, but that above 60 per cent. of the available capacity the growth of fruit was not materially decreased. The points plotted on figure 1 represent the rate of growth of fruit on all plots and for all periods during which the soil moisture was above 60 per cent. of the available capacity. These curves may be considered as representing the rate of growth during the year 1932 when water was not a limiting factor, or, so far as this study is concerned, as the "normal" rate of growth, and have been so designated.

TABLE I

SOIL MOISTURE CONSTANTS FOR VARIOUS DEPTHS IN THE FITCH, KLAMATH, AND MEDFORD EXPERIMENT STATION ORCHARDS

ORCHARD	0–1 FEET			1–2 FEET			2–3 FEET			0–3 FEET		
	FIELD CA-PACITY	WILT-ING POINT	AVAIL-ABLE CA-PACITY	FIELD CA-PACITY	WILT-ING POINT	AVAIL-ABLE CA-PACITY	FIELD CA-PACITY	WILT-ING POINT	AVAIL-ABLE CA-PACITY	FIELD CA-PACITY	WILT-ING POINT	AVAIL-ABLE CA-PACITY
	%	%	%	%	%	%	%	%	%	%	%	%
Fitch .	24.7	13.3	11.4	27.4	15.7	11.7	28.5	17.4	11.1	26.9	15.5	11.4
Klamath .	28.7	15.8	12.9	28.0	17.2	10.8	27.3	16.7	10.6	28.0	16.6	11.4
Medford Exp. Sta.	34.5	17.2	17.3	32.0	17.5	14.5	30.3	17.6	12.7	32.3	17.4	14.9

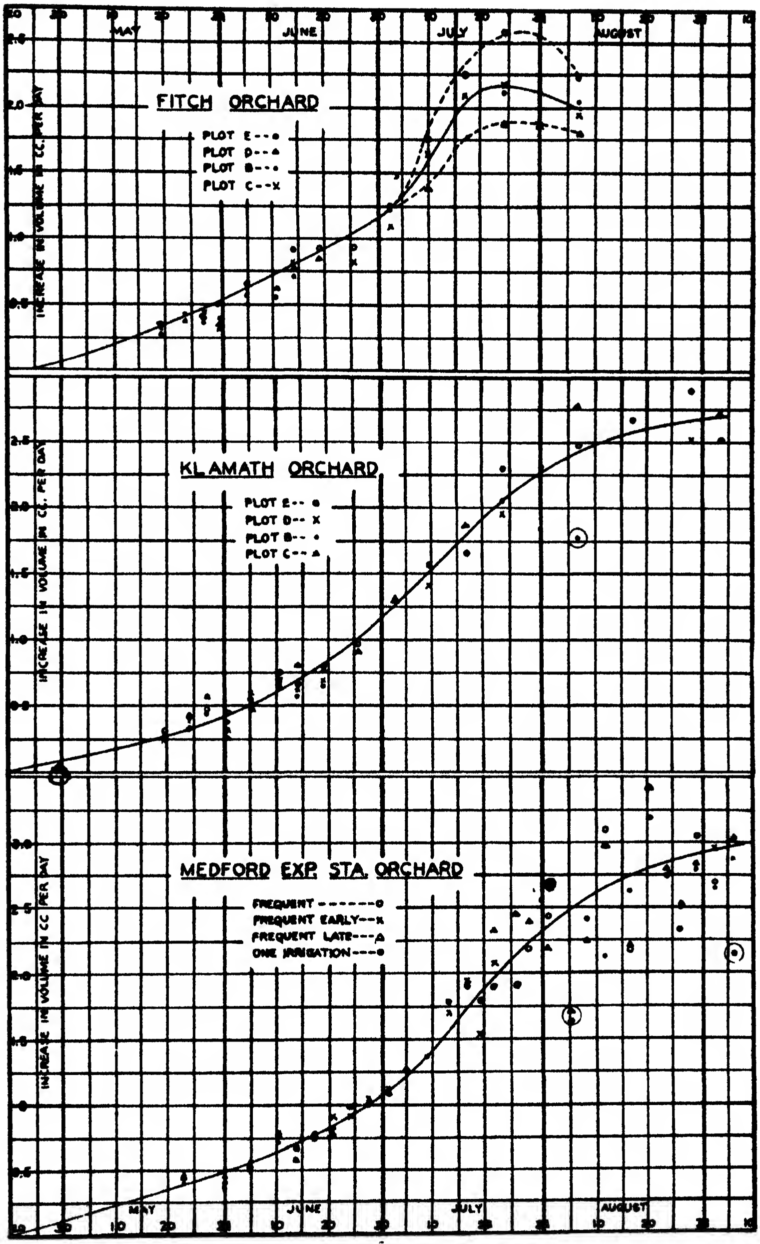

FIG. 1. Rate of growth of pears in cc. per day during 1932 in the Fitch, Klamath, and Medford Experiment Station orchards (the so-called "normal" rate of growth).

It is obvious that this curve will be affected by many factors other than the soil moisture conditions. The variety of fruit, climatic conditions, age and vigor of the trees, number of pears carried by the trees in comparison with their leaf area, and, perhaps, the size of trees, both above and below ground, have a bearing on the shape of the so-called "normal" growth curve. For these reasons separate curves have been plotted for each of the three orchards. In each orchard all treatments (including pruning, thinning, cultivating, etc.) except irrigation were as nearly alike as possible for all four plots. Within each orchard and for the one season it is believed that all conditions except soil moisture and, in one or two cases especially mentioned later, number of fruits per tree, are the same. It will be noted that on all three curves there are certain periods when the measured rate of growth is markedly slower or faster than is indicated by the smooth curves. Probably the most marked instance is for the period centering on May 31 or June 1. Many of these variations seem to be due to atmospheric conditions. In order to avoid introducing an error into the subsequent computations, the normal rate of growth for each period for the year 1932 is taken as the arithmetic mean of the rate for all the plots in which the moisture content was over 60 per cent. of the available capacity during that period, rather than the rate shown by the smooth curve.

It will be noted that the rate of growth curve for the Fitch orchard is broken into three parts late in the season. Study of the conditions on the plots in this orchard leads to the belief that the differences shown by plots E and D from the more central position of the plotted points for the other plots are due to differences in the leaf-fruit ratio, or the number of fruits borne by the trees in the various plots. Plot D apparently had a lower leaf-fruit ratio than plot E. The curve shown as a solid line is assumed to represent normal growth rate in the following discussion.

The curves of figure 2 show the deviation from the normal rate of growth of fruit for each orchard plotted against the soil moisture content. The difference between the measured rate of growth for each period for each plot and the normal rate of growth as just defined has been expressed as a percentage of the normal rate. This percentage deviation has then been plotted against the corresponding average available moisture content of the upper 3 feet of soil expressed as a percentage of the available capacity. These curves show a very definite relation between the rate of growth of the fruit of pear trees in this heavy soil and the amount of soil moisture in the root zone of the trees.

All three of these curves cross the line of normal growth at a moisture content of about 75 per cent. of the available capacity of the soil, and the maximum rate of growth is not attained below 80 or 85 per cent. The curves are very similar in shape and indicate that the effect of a shortage of

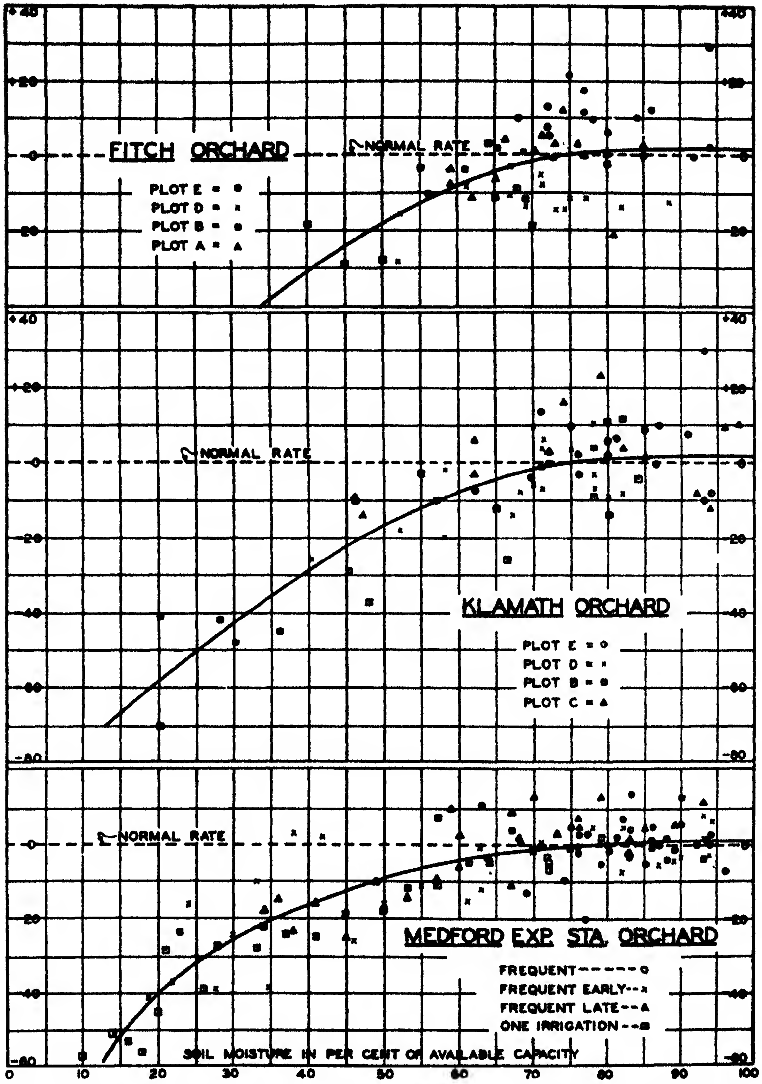

FIG. 2. Deviation from "normal" rate of growth as compared with percentage of available moisture present in the upper 3 feet of soil in the Fitch, Klamath, and Medford Experiment Station orchards in 1932.

soil moisture is similar in the three orchards with the two varieties of pears. When the soil moisture is reduced to 20–35 per cent. of the available capacity of the upper 3 feet, the rate of growth of fruit is 40 per cent. less than the normal rate of growth. This indicates that the lower limit of the range of soil moisture producing most rapid growth of pears in this soil is much higher than the wilting point.

These curves indicate that under the conditions of these experiments soil moisture is an important limiting factor when the moisture content of the upper 3 feet is less than about 75 per cent. of its available capacity. At higher moisture contents other factors appear to become limiting to such an extent as to overshadow the effect of soil moisture. Other factors undoubtedly have some effect under lower soil moisture conditions.

Figures 3, 4, and 5 show the moisture conditions in the plots during the

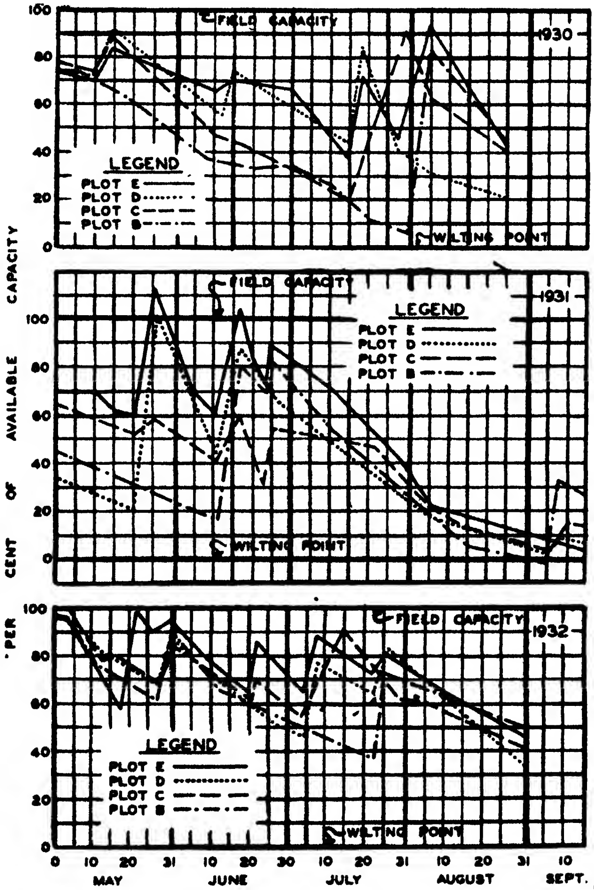

FIG. 3. Average moisture content of upper 3 feet of soil in the different plots of the Fitch orchard in 1930, 1931, and 1932, expressed as percentage of available capacity.

three years. The moisture content shown is the average for the upper 3 feet and is presented as the percentage of the available capacity present. Examination of original data shows that after an irrigation, the soil moisture drops more rapidly in the first foot than in either of the deeper feet, and that, in general, the second foot loses water more rapidly than the third

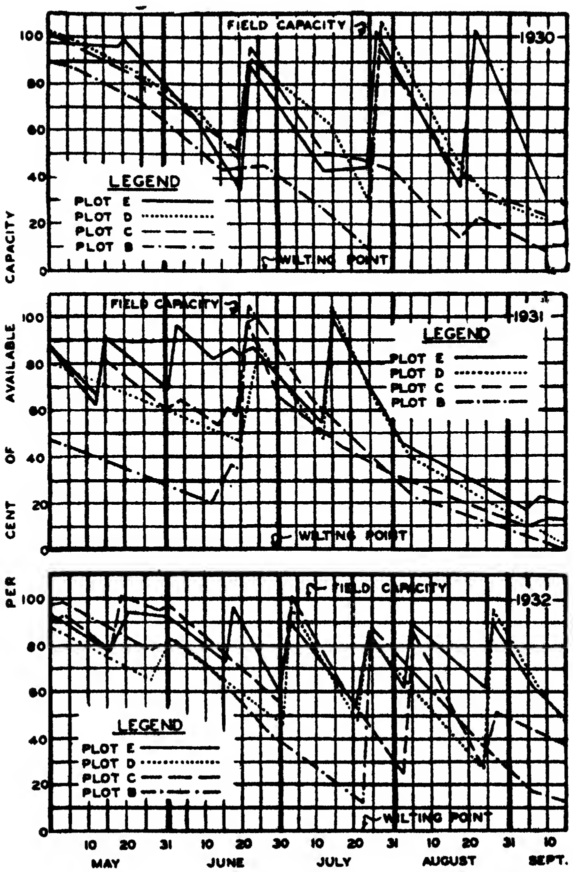

FIG. 4. Average moisture content of upper 3 feet of soil in the different plots of the Klamath orchard in 1930, 1931, and 1932, expressed as percentage of available capacity.

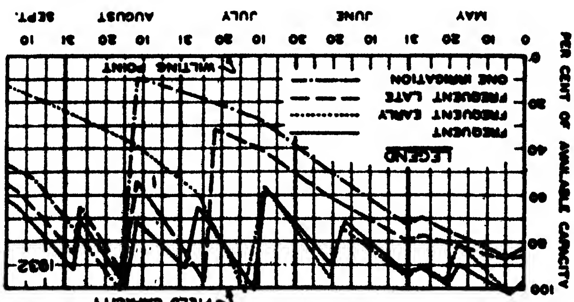

FIG. 5. Average moisture content of upper 3 feet of soil in the different plots of the Medford Experiment Station orchard in 1932, expressed as percentage of available capacity.

foot. In spite of this, in no case during the season of 1932 did the moisture content of the first foot of soil reach the wilting point.

In order to arrive at a check on the conclusion drawn here that the rate of growth of pear fruits is closely correlated with the soil moisture content of the upper 3 feet of soil, an estimate of the average size of the fruits harvested from these plots during all three years has been prepared. This method has been necessary because no measurements of the growth of the fruits were made during the growing seasons of 1930 and 1931.

Knowing the number of pounds of fruit to a packed box and also the number of fruits of each size to each packed box, it is possible to compute the number of pears of each size yielded by each plot. From data furnished by officials of local packing houses the volume of individual pears of the various sizes was determined. The average size of the individual fruits for each plot was then determined in the following manner. For each plot the total volume of fruit of each size was determined by multiplying the number of fruits by the volume of each fruit. The sum of the total volumes of fruits of each size then gave the total volume for the plot, and this figure divided by the total number of fruits of all sizes gave the average size of individual fruits for the plot. It is believed that this method gives an accurate estimate of the average size.

The data of figures 3, 4, and 5 on soil moisture and of figures 1 and 2 on the normal rate of growth and the effect of soil moisture on the rate of growth of the fruit make possible an estimate of the size of the average fruit at any time during the season. This has been done for each plot and for each season of our experiments. The moisture content in the upper 3 feet of soil in each plot for each day was determined from figures 3, 4, and 5. From the curves of figure 2 the deviation from the normal rate corresponding to the soil moisture content present each day was taken. This deviation was then applied to the normal rate for the day as shown on figure 1 and the estimated growth for the day determined. By summing up the growth for all the days the size of the fruit at picking time was estimated. Table II is a summary of the results obtained by the three methods: (1) measurement of the circumference of a great number of fruits in the field; (2) computation from the packing house records of yield and size of the fruit harvested from each plot; and (3) estimation from the soil moisture records and the curves of normal growth and deviation from normal growth as influenced by variation in soil moisture.

It is felt that the very close agreement between the size as estimated from the soil moisture data and that as determined directly from the fruit, either by direct measurement or by computation from yield records for the year 1932, is remarkable. The volumes computed from yield data in the Fitch orchard are not directly comparable with the volumes secured by the

TABLE II

SIZE OF PEAR FRUITS ON ALL PLOTS AND ALL SEASONS: AS MEASURED (COLUMN 1); AS COMPUTED FROM YIELD RECORDS (COLUMN 2); AND AS ESTIMATED FROM SOIL MOISTURE DATA (COLUMN 3)

YEAR	PLOT E			PLOT D			PLOT C			PLOT B		
	1	2	3	1	2	3	1	2	3	1	2	3
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
	Fitch orchard											
1930		107.1*	105.1		101.5*	91.1		104.0*	88.1		98.3*	63.0
1931		144.7*	88.4		131.5*	72.3		127.0*	74.9		125.8*	69.9
1932	120.4	141.8*	111.3	93.6	125.8*	104.6	105.0	131.0*	105.6	97.2	134.7*	97.8
	Klamath orchard											
1930	.	148.0	170.0		137.2	155.2		114.0	120.6	...	120.0	133.6
1931	....	130.0	130.3		132.0	121.7		98.8	104.4	.	96.0	95.0
1932	184.3	175.0	178.3	161.0	162.0	164.7	181.9	169.0	155.3	121.5	122.0	133.4
	Medford Experiment Station orchard											
	PLOT FREQUENT			PLOT FREQUENT EARLY			PLOT FREQUENT LATE			PLOT ONE IRRIGATION		
1932	180.4	180.0	186.3	157.6	158.5	157.7	180.6	180.0	179.2	143.2	149.5	146.2

* In the Fitch orchard the field measurements of fruit and the estimates from soil moisture conditions were made only up to the time of the first picking. As a part of the fruit was left on the trees and grew rapidly before the second picking, the size of fruits computed from the yield and size data is considerably larger than that shown by the other methods.

other two methods. The greatest discrepancy in comparable data is in the case of plot C in the Klamath orchard. A probable explanation for the marked difference between the measured volume and the volume computed from yield data in this plot is that growth measurements were made on the lower branches where the leaf-fruit ratio was larger than on the upper branches where the bulk of the crop was carried. The fact that both of these volumes are large as compared with the volume estimated from soil moisture is explained by the small number of fruits carried by the trees in this plot. It seems to be generally true that, all other things being equal, a tree with a small number of fruits will produce fruits of a larger size than will the same tree with a large number of fruits. In this case the measured volume is 17 per cent. larger and the computed volume is 8.8 per cent. larger than the estimated volume. In no other case in 1932 is the estimated volume more than 10.5 per cent. different from the comparable volume determined by other methods.

Since it was necessary to apply the so-called normal rate of growth found in 1932 to the moisture conditions found in the plots in 1930 and 1931, it is not to be expected that as close an agreement will be found in these years. Again the figures for the Fitch orchard are not directly comparable. Except for the reversal of plots C and D, which reversal is only a matter of 3 or 4 cc., the order of the sizes in the plots is the same by computation from yield data and estimation from soil moisture. The much larger sizes of fruit determined from yield data as compared with the expected sizes computed from moisture conditions in the Fitch orchard in 1931 are due to the fact that the fruit in this orchard was heavily thinned that season in anticipation of a water shortage. In the Klamath orchard the maximum difference between the two methods is 13 per cent. These data furnish convincing proof that the effect of soil moisture deficiency is reasonably well defined by the curves of figure 2.

Discussion

As pointed out by ALDRICH and WORK (1), such results on heavy soils seem to lead to conclusions contrary to those arrived at by some other workers using lighter soils. HENDRICKSON and VEIHMEYER (4) conclude that "trees either *have* readily available moisture or *have not*." BECKETT, BLANEY, and TAYLOR (3), working with citrus and avocado trees, reach the somewhat similar conclusion that "moisture is as readily available when the moisture content is one-third or two-thirds of the way between field capacity and the wilting point as it is in the thoroughly moistened soil after irrigation."

Three reasons either singly or in combination may account for the slowing down of the growth of fruit in our experiments as the quantity of water held in the root zone decreases.

(1) Perhaps soil moisture becomes actually less available to the roots of the trees as the thickness of the moisture film decreases. If this is the explanation it must become effective at film thicknesses nearly as great as those present when the moisture is held in approximate equilibrium between gravitational and capillary forces and become progressively more marked as the moisture content approaches the wilting point. The data reported by SEKERA (5) seem to support this explanation.

(2) The growing root tips or root hairs which are actively extending into moist soil may not be able to advance rapidly enough to develop sufficient water to meet the needs of the trees or they may not be able to penetrate all parts of the soil mass. When, just after an irrigation, the moisture content of the soil of the root zone is at or near the field capacity, each growing root may be supposed to be in contact with a film of available water. As the water in contact with the active root tips or hairs is exhausted, it is at first relatively easy for most of the growing points to advance and thus secure more water. It is conceivable, however, that more and more of the growing points may for one reason or another find it impossible to follow the retreating water films, and thus cease to function. It seems reasonable to suppose that this condition would be much more apt to occur in very dense and fine-textured soils such as these. As a portion of the growing roots become inactive, those remaining would become progressively less able to supply the quantity of water required to keep the fruit growing at the maximum rate.

(3) It is inconceivable that the roots of these trees can come in direct contact with every soil particle and its enveloping film of water. If this be true, there must be some movement of water from those portions of the soil not in direct contact with active root tips or hairs toward these roots before the moisture content of the soil mass can be reduced to the wilting point. The distance through which this movement will take place will depend on the completeness with which the roots are able to occupy the soil. In heavy soils such as these, the soil moisture gradient toward the active roots must be rather steep if appreciable movement is to take place. The moisture supply to the tree may then be fixed, not by the rate at which roots can take up water but by the rate at which the water can move through the soil to the roots.

It will be evident that under either of the two latter conditions the moisture content of the soil immediately in contact with active roots may be reduced to the wilting point, while the soil at some distance (perhaps a very small one) may have a much higher content of moisture. Now the moisture content of a soil sample taken by ordinary methods would be a sort of average of that held by the soil adjacent to the root hairs and that held at some distance from any active root. The moisture content disclosed by

sampling and the actual *average* moisture content of a portion or all of the root zone might then be well above the wilting point, while the actual moisture content of the soil adjacent to a portion (or in extreme cases, all) of the active roots might be down to the wilting point.

It is the opinion of the writers that some combination of the slow movement of water through this tight soil and the relatively sparse root population in the soil accounts for the effect on the growth of fruit of comparatively small changes in the average moisture content of the soil in the root zone.

Summary

1. The field capacity of the soil in the three orchards included in the test varied from 24.7 to 34.5 per cent., the wilting point from 13.3 to 17.6 per cent., and the available capacity from 10.6 to 17.3 per cent.

2. The rate of growth during those periods and on those plots when and where the soil moisture was more than 60 per cent. of the available capacity is designated the normal rate of growth and is shown graphically.

3. The deviation of the rate of growth from this normal, expressed as a percentage of the normal, is plotted against the soil moisture in the upper 3 feet.

4. The curve resulting from this plotting shows a very close correlation between moisture content and rate of growth of fruit.

5. The sizes of fruit as measured in the field, as computed from yield records, and as estimated from soil moisture data check remarkably well.

6. It is believed that the data furnish definite proof that the rate of growth of pear fruits is markedly affected by comparatively small variations in the moisture content of the soil of the root zone, even when the moisture content is well above the wilting point.

7. The data indicate that the growth of the fruit of pear trees on this heavy soil is reduced whenever the soil moisture in the major portion of the root zone is reduced below 70 per cent. of the available capacity.

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COLORIMETRIC DETERMINATION OF CAROTENE IN PLANT TISSUE[1]

WALTER C. RUSSELL, M. W. TAYLOR, AND D. F. CHICHESTER

(WITH TWO FIGURES)

Introduction

The discovery by BORODIN (2) in 1883 that the carotenoid pigments could be separated into alcohol soluble and petroleum ether soluble groups has been the basis for all the procedures that have been described for the determination of carotene and other carotenoid pigments. ARNAUD (1), in 1887, extracted dry plant tissue with petroleum ether and used a colorimetric method for the estimation of the amount of pigment present, a carotene solution being employed as a standard. No attempt was made to separate carotene from other yellow pigments and the purity of the carotene standard was not given. In 1913, MONTEVERDE and LUBIMENKO (9) reported a spectro-colorimetric method for the estimation of the pigments of green leaves, and in the same year WILLSTÄTTER and STOLL (17) presented a method for the determination of carotene and xanthophyll which has served as the starting point for all subsequent modifications. The latter method consists essentially of acetone extraction of plant tissue, saponification of chlorophyll, separation of the carotenoids by means of petroleum ether and aqueous methyl alcohol, and the colorimetric estimation of the pigments. A petroleum ether solution of carotene or an aqueous solution of potassium dichromate served as a colorimetric standard. COWARD (3), in 1926, modified the procedure by making the first step the decomposition of chlorophyll, which was followed by extraction with petroleum ether and the separation of carotene from xanthophyll by aqueous methyl alcohol. The use of diethyl ether in addition to acetone in the extraction of plant tissue was introduced by SCHERTZ (12) in 1928. In 1923 he described a method (13) for the spectrophotometric estimation of carotene. SPRAGUE and SHIVE (15), 1929, employed the method as modified by SCHERTZ (12), except that they used petroleum ether rather than diethyl ether as a solvent for the carotenoids. These investigators (15) and SPRAGUE and TROXLER (16) developed a color standard of dye solutions for use in colorimetric readings. Pyridine has been employed by SMITH and SMITH (14) for the extraction of small quantities of fresh fruit, the pigments being transferred to petroleum ether. Recently, KUHN and BROCKMANN (6) have described the use of petroleum ether and methyl alcohol in the extraction of plant tissue and the subsequent separation into

[1] Journal Series paper of New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

petroleum ether and aqueous methyl alcohol phases. After these steps, saponification with alkali in the petroleum ether phase is followed by a further partition between petroleum ether and 90 per cent. methanol. The use of suitable absorption agents allows the separation of α and β carotene from lycopin in the petroleum ether phase. A solution of azobenzene was used as the colorimetric standard.

The original objective of this investigation was the correlation of the carotene content of plant tissue with vitamin A potency, determined biologically, and with the biological response when isolated carotene was fed. Hence, an accurate method for the determination of carotene which would give consistently reproducible results was necessary. In our hands the method as modified by SPRAGUE and SHIVE (**15**) when used for a series of determinations on the same sample of dried green plant tissue did not give results which agreed within the experimental error allowable in the work projected; therefore a critical study was made of the original WILLSTÄTTER and STOLL (**17**) procedure as modified by SPRAGUE and SHIVE.

Analytical procedure

PREPARATION OF SAMPLE

As the first step in the analytical procedure it was necessary to ball-mill the dried plant tissue until a fine powder was obtained. It has been our practice with artificially dried alfalfa to reduce the chopped product to a coarsely divided state by means of a Wiley mill and then to subject it to ball-milling.

In the case of fresh plant tissue (alfalfa), the whole plant was cut into 5–10 mm. pieces with a pair of large shears as quickly as possible after harvesting and the samples for analysis weighed immediately.

Methods of drying of plant tissue which do not cause a loss of carotene require further investigation before definite recommendations can be made. The work of the present investigation was done largely with machine dried and field dried alfalfa. Machine dried material which had been subjected momentarily to an initial temperature as high as 650° to 750° C., and then to lower temperatures until dry, was found to contain practically the same amount of carotene as fresh alfalfa. On the other hand, the slow drying of silage in a vacuum desiccator over sulphuric acid at room temperature resulted in a loss of 25 per cent. of the carotene present in the fresh material. These findings suggest that a high initial temperature destroys enzymes which might be active in the destruction of carotene at lower temperatures.

EXTRACTION OF PIGMENTS

The sample of material weighed for analysis should contain about 0.2 mg. of carotene so that final dilution to 100 cc. will give a solution contain-

ing about 0.002 mg. per cc. for use in the colorimeter. If information concerning the approximate percentage of carotene is not available, it is advisable to make preliminary analyses. With samples very low in carotene, better results have been obtained by using a smaller sample than the amount which would contain 0.2 mg., and a correspondingly smaller final volume for the colorimetric comparison. In this investigation the size of samples varied from 1 to 25 gm.

Since carotene was the only pigment to be determined, it was not necessary to isolate chlorophyll and xanthophyll. Dried alfalfa was extracted directly with petroleum ether, because it had been found, in the case of this material, to extract carotene completely while removing only a small amount of the chlorophyll and xanthophyll. In most instances the extraction was carried out by allowing the sample to stand in petroleum ether for 72 hours, with occasional stirring, although for some types of tissue a shorter period of time was sufficient. The volume of petroleum ether varied from 125 to 250 cc., the larger amount being necessary for large samples low in carotene. It was found convenient to carry out the extraction in 250-cc. centrifuge bottles and to separate the plant tissue from the solvent at the end of the extraction period by centrifuging. The finely divided samples did not lend themselves well to filtration, and less solvent was retained by the plant tissue when the separation was made with the centrifuge. The liquid layer was poured or siphoned into a 1-liter separatory funnel, a filter being used as a precaution against the introduction of any of the plant tissue. The extracted tissue was washed with several 50-cc. portions of petroleum ether, five portions with stirring and centrifuging after each addition usually being sufficient. In dealing with new material or different lots of the same material, it was found advisable to test for completeness of extraction by repeating the analytical procedure on the sample which had already been extracted. The amount of carotene obtained by this second extraction determined the length of the extraction period and the number of extractions to be used.

Samples of fresh plant tissue were triturated with sand under acetone and then allowed to stand under the solvent for 48 hours. After decantation through a filter, the residue was washed with several portions of fresh acetone. If the extraction is not complete, as indicated by the removal of all green color from the material, it is necessary to grind the sample further with sand and repeat the extraction. To carry out the remainder of the analysis, the pigments were transferred from acetone to petroleum ether by adding about 400 cc. of the latter solvent to the acetone solution in a separatory funnel. The acetone was then washed out with several 300- to 400-cc. portions of water. Care must be taken in this operation to avoid excess shaking on account of the tendency to form an emulsion.

The present study has been concerned almost entirely with alfalfa and spinach plant tissue, and the methods of extracting the plant pigments just described may not apply equally well to other plant tissues. As noted before, SMITH and SMITH (**14**) have employed pyridine for the extraction procedure. Preliminary studies in this laboratory show that pyridine removes from dried carrots and yellow corn an additional amount of carotene, even after they have been thoroughly extracted with petroleum ether. The use of pyridine with alfalfa, however, which had already been extracted with petroleum ether, did not reveal the presence of additional carotene; hence in the application of this method to plant tissue other than alfalfa, special attention should be given to the extraction procedure.

SEPARATION OF PIGMENTS

In the case of both dry and fresh samples it is necessary to remove chlorophyll and xanthophyll from the petroleum ether solution. To remove xanthophyll, the solution, having a volume of approximately 400 cc., was shaken in a 1-liter separatory funnel with 50-cc. portions of 89 per cent. methyl alcohol for 2-minute periods. This process was continued until the methyl alcohol layer was only very slightly colored. Usually six extractions were sufficient and at the end of this time only a small amount of xanthophyll still remained in the petroleum ether. However, these last traces of xanthophyll were removed during the process for the removal of chlorophyll. The procedures which involved shaking in a separatory funnel, were facilitated by the use of a shaking machine which had been modified so that it would hold four 1-liter funnels in a horizontal position. Figures 1 and 2 provide a description of the machine. The stopcocks of the funnels were lubricated with stopcock grease and, although there was a slow dissolving of it by the petroleum ether, renewal between every two or three analyses prevented any leakage. The glass stoppers were held so firmly by the pressure of the clamps that there was no leakage. To avoid the possibility that they might become stuck, however, they were lubricated with a soft soap, made by warming together 100 parts of Ivory soap, 100 of water, and 15 of glycerol. This was preferable to stopcock grease which quickly dissolved from around the stopper. It was necessary of course to use lubricants which did not impart a color to the solvents.

Although WILLSTÄTTER and STOLL (**17**) prescribed the use of one portion of 85 per cent. methyl alcohol, one of 90 per cent., and two or more of 92 per cent., it has been the experience in this laboratory that any solution of methyl alcohol greater than 90 per cent. by volume will emulsify if shaken vigorously with petroleum ether; whereas emulsion formation was avoided entirely if the concentration of alcohol was 88 to 89 per cent. by volume.

Chlorophyll was removed by shaking thoroughly the petroleum ether solution with 25 per cent. potassium hydroxide in absolute methyl alcohol. Successive 25-cc. portions of the alcoholic potassium hydroxide were used until no further color was removed. The complete removal of chlorophyll is important at this point since chlorophyll in very low concentrations will impart a slightly yellow color not unlike that due to carotene. In testing for the complete removal of chlorophyll, it was found convenient to have

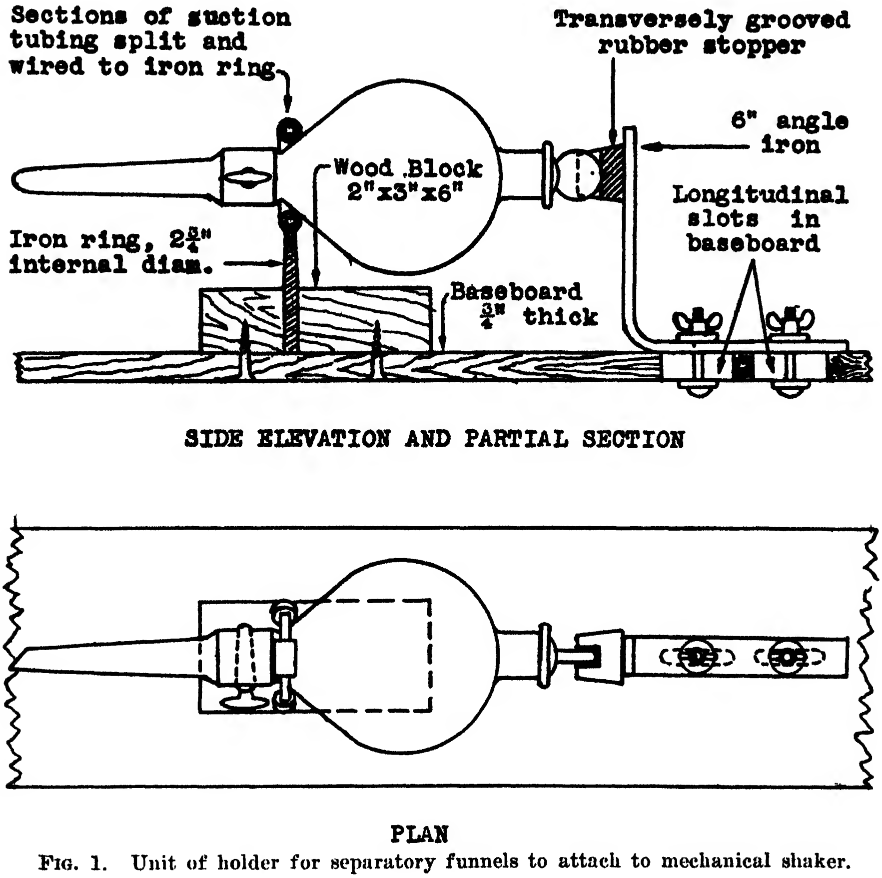

FIG. 1. Unit of holder for separatory funnels to attach to mechanical shaker.

for comparison a series of very dilute color standards in tubes prepared by diluting the standard potassium dichromate solution. The following concentrations, in terms of carotene equivalents, were employed: 0.01, 0.004, 0.002, 0.001, 0.0004, 0.0002, and 0.0001 mg. per cc. The removal of chlorophyll was considered to be complete when the alcoholic potassium hydroxide extract had a color value equivalent to less than 0.0001 mg. of carotene per cc.

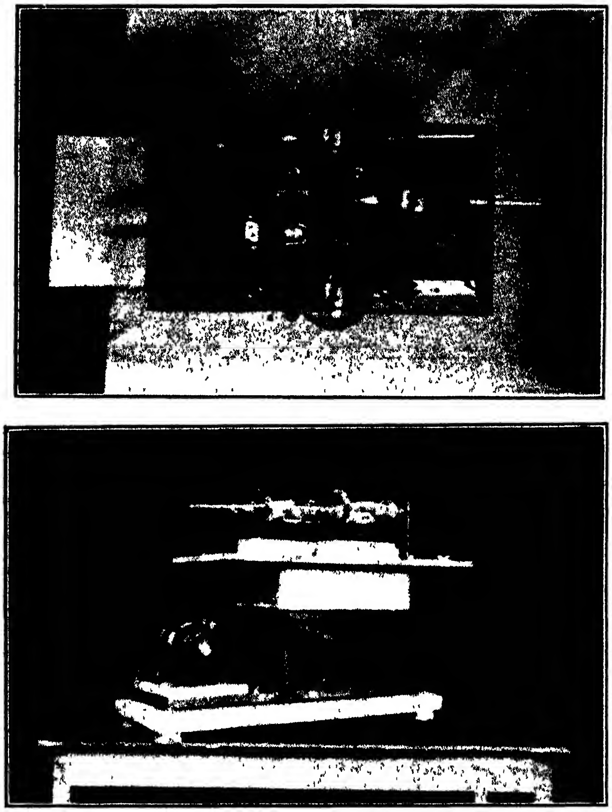

Fig. 2. Assembled apparatus for shaking separatory funnels.

After removal of chlorophyll and xanthophyll, the petroleum ether solution was shaken with 50 cc. of 89 per cent. methyl alcohol, followed by 100 cc. of distilled water in order to remove traces of base. The resulting petroleum ether solution, which contained only carotene, was transferred to a 400-cc. beaker and evaporated to a suitable volume, this process being carried out *in vacuo* at 40°–45°. For this purpose a vacuum desiccator, continuously evacuated with a water pump, was used in a constant temperature oven. When a suitable volume had been obtained, the petroleum ether solution was transferred quantitatively to a volumetric flask, made up to volume with petroleum ether,[2] and compared in a Buerker colorimeter with the standard solution of potassium dichromate, described in a following section.[3]

[2] The use of a high boiling petroleum ether, "Skelleysolve," has been suggested by Prof. R. A. Dutcher, Pennsylvania State College, in a private communication.

[3] Dr. E. S. Miller (see addendum) determined the carotene content of a petroleum ether solution prepared by this procedure by spectral analysis and found it to contain 97 per cent. of the carotene content determined colorimetrically in this laboratory.

Reagents

The acetone and potassium hydroxide were the usual c.p. grade. The petroleum ether was a commercial grade, b.p. 30° to 60°, obtained in 50-gallon drums and redistilled before using. Methyl alcohol was purchased as refined methanol and was sufficiently pure, as indicated by the fact that no color developed in its solution of potassium hydroxide in two weeks. The presence of a cloudiness, presumably potassium carbonate, in the alcoholic solution makes it difficult to determine whether a colorless extract has been obtained in the analytical procedure. The cloudiness was easily removed by centrifuging.

It was found to be economical to recover by distillation the methyl alcohol solution used to remove xanthophyll and to adjust the distillate to the original 89 per cent. concentration.

Colorimetric procedure

Both spectrophotometric and colorimetric methods have been used by various investigators in estimating the concentration of carotene in the petroleum ether solution obtained by the analytical procedure. The former method has been described by Schertz (**12**) and by Ferrari and Bailey (**4**). However, the error involved in the isolation of carotene is probably as great as that inherent in the colorimetric method, and therefore there seems to be no advantage in the use of the spectrophotometric procedure for which greater accuracy has been claimed (**12**). Recently methods have been proposed, such as that by Oltman (**10**), which involve the measurement of the intensity of transmitted light by means of a photoelectric cell and aim to eliminate the subjective factor inherent in colorimetric readings. To the best of our knowledge, however, methods of this type have not been brought to a wholly satisfactory stage of development.

The reported instability of carotene and the necessity of dissolving it in volatile liquids render it unsuitable as a colorimetric standard. As secondary standards, Willstätter and Stoll (**17**), Palmer (**11**), and Coward (**3**) have made use of potassium dichromate; whereas Sprague and Shive (**15**) availed themselves of a mixture of the dyes Orange G and Naphthol Yellow. Kuhn and Brockmann (**6**) have employed azobenzene. During the early part of this investigation the dye standard described by Sprague and Shive was used, but later it became evident that it possessed no advantage over potassium dichromate, and that the latter was preferable because of its greater uniformity of color and stability in solution.

Both the Orange G-Naphthol Yellow and the potassium dichromate solutions were evaluated against standard carotene solutions. Crystals of the pigment were isolated from dried alfalfa and dried carrots by a process

which is the same in principle as the analytical procedure for dried plant tissue described in detail in previous sections. Three lots of carotene were prepared and the following quantities weighed to the fifth place by the method of swings: carrot carotene lot 1, 39.8 mg.; carrot carotene lot 2, 22.3 mg.; and alfalfa carotene, 18.1 mg. Each sample was brought into solution in slightly less than 200 cc. of petroleum ether with warming to not above 50°, after which the volume was made up to 200 cc. Dilutions of the carotene solutions were prepared which were suitable for comparison with the secondary standards in the region of 10 mm. on the scale of the Buerker colorimeter. The secondary dye standard described by SPRAGUE and SHIVE (**15**) was prepared by diluting 3.4 cc. of 0.5 per cent. Naphthol Yellow and 0.5 cc. of 0.5 per cent. Orange G to 1 liter. For the standard potassium dichromate solution, a concentration of 0.036 per cent. was used because this solution matched the dye standard very closely. Table I shows the carotene values of the two secondary standards. The carotene equivalent of the potassium dichromate solution was adopted as 0.00208 mg. per cc. (See addendum.)

TABLE I

CAROTENE VALUES OF THE SECONDARY STANDARDS

SOURCE OF CAROTENE	MELTING POINT, CORR.	DYE STANDARD. CAROTENE EQUIVALENT PER CC.	POTASSIUM DICHROMATE STANDARD. CAROTENE EQUIVALENT PER CC.
	°C.	*mg.*	*mg.*
Carrot 1	175°–177°	0.00215	
Carrot 2	171.5°–175.5°	0.00227	0.00205
Alfalfa	176.5°–179°	0.00228	0.00210
	Average	0.00223	0.00208

Recent investigations have revealed the existence of two forms of carotene, α and β (**5, 8**) and possibly a third, γ carotene (**7**). These findings raise the question as to whether the several forms are colorimetrically equivalent. KUHN and BROCKMANN (**6**) have reported that in concentrations of the order of 0.00235 mg. per cc., the α and β forms appear to be equal; but that in higher concentrations there is a difference expressed by the proportion α carotene : β carotene :: 1.0 : 1.2. The concentration at which they are equivalent is of the same order of magnitude as that employed throughout the present investigation, the 0.036 per cent. potassium dichromate standard solution being equivalent to 0.00208 mg. of carotene per cc. Earlier workers used standards which had a carotene value

several times as high as that just described. Thus WILLSTÄTTER and STOLL (17) and COWARD (3) employed a 0.2 per cent. potassium dichromate solution.

In the use of a colorimetric standard, it is necessary to determine any deviation from Beer's law for a given concentration of standard and type of colorimeter. PALMER (11) has plotted the data obtained by WILLSTÄTTER and STOLL when a Wolff colorimeter was used, and notes that a Dubosq or Kober colorimeter should serve as well. COWARD (3) has prepared a curve for the use of carotene solutions with the Hellige colorimeter. Of several types of colorimeters available, the Buerker was found to be the most satisfactory, probably because it compensates for the use of different solvents in the standard and in the unknown.

To determine the relationship between observed and calculated values over a range of 5 to 20 mm. when the Buerker colorimeter was used, a series of solutions of known concentrations were prepared from alfalfa carotene and from lot 2 of carrot carotene. The solutions were related as 1.5x, x, 0.77x, and 0.57x in the alfalfa carotene series and in the carrot carotene series the relationship was 2x, x, 0.65x, and 0.5x. In the case of each series, concentration x was selected so as to match the standards as nearly as possible at equal depths, that is at 10 units on the scale. The various concentrations of carotene were read in the colorimeter against the secondary standards set at the fixed depth of 10 mm. In table II the observed and calculated colorimeter readings are shown. The latter were calculated upon the assumption that there was no deviation from Beer's law.

Although the differences between the observed and calculated values in table II in most instances fall within the error of colorimetric observation, yet they increase and are positive in character, as the colorimeter reading becomes greater, indicating a deviation from Beer's law. These results and experience with the method in this laboratory lead to the recommendation that readings with the Buerker colorimeter should be made between 7 mm. and 14 mm., with the standard set at 10 mm.

The volatility of the low-boiling petroleum ether used necessitated special precautions in the preparation of solutions of known concentration. To avoid errors from the evaporation incident to pouring, dilution of the most concentrated solution of each series was carried out by measuring the required amounts from a 100-cc. burette into 100-cc. volumetric flasks. The burette was filled from the bottom by suction rather than by pouring, and tilting or agitation of the bottle of concentrated solution was avoided as much as possible. After the preparation of each dilution, the solution remaining in the burette was read colorimetrically and was found not to have undergone any change in concentration. The transfer of the various

TABLE II

Relation of concentration to depth of color

Relative concentration of carotene	Dye standard trial 1			Dye standard trial 2			Dichromate standard		
	Observed colorimetric reading	Calculated colorimetric reading	Difference	Observed colorimetric reading	Calculated colorimetric reading	Difference	Observed colorimetric reading	Calculated colorimetric reading	Difference
Carrot carotene	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Lot 2									
2 x	4.9	5.0	+ 0.1	4.8	4.8	0.0	5.2	5.1	− 0.1
x	9.9			9.6			10.3	.	.. .
0.65 x	15.0	15.2	+ 0.2	14.5	14.7	+ 0.2	15.7	15.8	+ 0.1
0.50 x	19.0	19.8	+ 0.8	18.6	19.1	+ 0.5	20.0	20.6	+ 0.6
Alfalfa carotene									
1.5 x	6.4	6.4	0.0	6.8	6.8	0.0	7.1	7.0	− 0.1
x	9.5			10.2			10.5	. .	..
0.77 x	12.4	12.4	0.0	13.3	13.2	− 0.1	13.6	13.7	+ 0.1
0.57 x	16.3	16.7	+ 0.4	17.6	17.9	+ 0.3	18.1	18.5	+ 0.4

solutions to the colorimeter cup was made by pipetting rather than by pouring.

As has already been stated, the dye standard of SPRAGUE and SHIVE (15) was employed in the early stages of this investigation. That the results are essentially the same with both standards is evident from table II, and therefore the dichromate standard was adopted on account of its greater stability and reproducibility. When the dye standard was developed several years ago by SPRAGUE and SHIVE, it was evaluated against a carotene solution, prepared from plant tissue, whose carotene content was determined with a spectrophotometer through the courtesy of Dr. F. M. SCHERTZ of the United States Department of Agriculture. The carotene value of the standard was placed at 0.00189 mg. per cc. As will be noted in table I, the average carotene value obtained by comparison with solutions of three different samples of crystalline carotene is 0.00223 mg. per cc. In view of the more recent findings with regard to carotene and because the solutions used in the present investigation were prepared from crystalline carotene, the later value has been adopted as the carotene value of the dye standard in preference to the earlier one.

Comparison of petroleum ether with aqueous acetone extraction

In the early stages of this study 85 per cent. aqueous acetone, recommended by WILLSTÄTTER and STOLL (17), was used for the extraction of dry plant tissue, prior to the transfer of the pigments to petroleum ether. ARNAUD (1) had previously used direct extraction of the pigments with the latter solvent, and this step in the procedure was developed further in this investigation. A comparison of the results obtained by the two procedures is presented in table III, in which it is evident that good agreement prevails between the two methods. Hence direct extraction of dry plant tissue with petroleum ether was adopted because the procedure could be shortened without sacrifice of accuracy.

TABLE III

COMPARISON OF PETROLEUM ETHER WITH AQUEOUS ACETONE EXTRACTION

SAMPLE	CAROTENE CONTENT, PETROLEUM ETHER EXTRACTION	CAROTENE CONTENT, AQUEOUS ACETONE EXTRACTION
	%	%
Alfalfa 45	0.0039	0.0040
	0.0039	0.0044
	0.0040	0.0040
	0.0040	
Spinach 44	0.0167	0.0175
	0.0167	0.0175

TABLE IV

LOSS OF CAROTENE DUE TO MANIPULATION

SAMPLE	CAROTENE IN SAMPLE OF TISSUE	CAROTENE ADDED	TOTAL CAROTENE, CALCULATED	CAROTENE FOUND	CAROTENE LOST	LOSS AS PERCENTAGE CAROTENE ADDED
	mg.	mg.	mg.	mg.	mg.	%
Alfalfa 45	0.188*	0.183	0.371	0.362	0.009	4.9
		0.183	0.371	0.362	0.009	4.9
	0.163*	0.186	0.349	0.340	0.009	4.8
	0.143	0.178	0.321	0.314	0.007	4.9
Spinach 44	0.158*	0.182	0.338	0.333	0.005	2.8
		0.182	0.338	0.333	0.005	2.8
	0.161*	0.182	0.343	0.335	0.008	4.5
		0.182	0.343	0.338	0.005	2.7
	0.122	0.178	0.300	0.293	0.007	5.7

* Average of duplicate determinations.

Loss of carotene due to manipulation

To determine whether there was a loss of carotene during the analytical procedure, a solution of crystalline carotene was prepared so that 100 cc. contained an amount of carotene which was approximately the same as that in a sample of plant tissue used for colorimetric analysis. One hundred cc. of this solution was added to a weighed portion of dry plant tissue and the total carotene content determined. At the same time carotene was determined in another sample of the dried plant tissue. The difference between the amount of carotene determined in the prepared sample and that calculated to be present is the amount of carotene lost. In table IV these data and the percentage of pigment lost are shown. Losses of the order of 5 per cent. demonstrate that the destruction of carotene during the analytical procedure is slight and is probably within the range of experimental error.

Application of analytical procedure

In table V there is shown a series of results obtained upon samples of

TABLE V

ANALYTICAL RESULTS

Alfalfa 45		Alfalfa 61		Alfalfa 62	
Date	Carotene	Date	Carotene	Date	Carotene
	%		%		%
1/16/32	0.0038	4/27/32	0.0062	4/11/32	0.0022
	0.0041		0.0061		0.0023
1/26/32	0.0039	5/28/32	0.0060	4/24/32	0.0024
	0.0039				0.0024
	0.0040				
	0.0039				
		6/ 2/32	0.0063		
2/ 5/32	0.0041	7/19/32	0.0059	5/27/32	0.0021
	0.0041		0.0057		0.0022
		8/ 8/32	0.0058	7/19/32	0.0021
			0.0055		0.0022
Mean	0.0040		0.0059		0.0022
Coefficient of variation	0.96		1.5		1.92

alfalfa over a period of several months which demonstrate that consistently reproducible results can be obtained with the method described. The material was stored at 0° ± 5°.

Summary

1. The method originally described by WILLSTÄTTER and STOLL, and since modified by others, has been examined critically and revised so as to permit a more complete separation of carotene from other plant pigments.

2. A potassium dichromate solution, of lower color value than that employed by previous investigators, has been evaluated against carotene preparations of known purity and can be used when either the α or β or both forms of carotene are present. (See addendum.)

3. The revised method permits a recovery of 95 to 97 per cent. of added carotene.

Addendum.—After this paper was accepted for publication, spectral methods for the determination of α and β carotene were brought to a successful completion by DR. E. S. MILLER, National Research Council Fellow, Department of Chemistry, University of Chicago. (Plant Physiol. 9: 681. 1934.)

Also the development of more refined procedures made possible a supply of α and β carotene. A small quantity of each of these isomers was purchased from the S.M.A. Corporation, Cleveland, Ohio, and portions of the same lot of each were sent to DR. E. S. MILLER and to his laboratory. MILLER determined the purity of the two isomers and found it to be 97 per cent. in each case. The colorimetric value of the potassium dichromate standard was evaluated against each of the isomers in this laboratory. Three separate weighings and solutions were made of each of the isomers. The values observed for the dichromate standard in the case of each of the isomers and their averages are shown in table VI. Although the values when compared with α carotene are generally

TABLE VI

α AND β CAROTENE EQUIVALENTS OF THE POTASSIUM DICHROMATE STANDARD

CAROTENE SAMPLE	PURITY	CAROTENE EQUIVALENT PER CC. OF POTASSIUM DICHROMATE STANDARD		
		OBSERVED VALUES		CORRECTED TO 97% PURITY
	%	*mg.*		*mg.*
Alpha	97	0.00215 0.00211 0.00218	Av. 0.00215	0.00209
Beta	97	0.00209 0.00207 0.00211	Av. 0.00209	0.00203
				Average 0.0026

slightly higher than those for the β form, the difference between the averages of the two sets of readings is within the error commonly accepted for colorimetric work. Therefore it was considered advisable to average the corrected values and to adopt 0.00206 mg. per cc. as the carotene equivalent for the 0.036 per cent. potassium dichromate solution. This value is only slightly different from that originally adopted, 0.00208 mg. per cc. The use of a potassium dichromate standard of the above concentration will permit the

determination of the carotene content of plant tissue when only one or both of the isomers are present. We wish to express to Dr. Miller our appreciation of his critical examination of our paper and of his cooperation in the making of these determinations.

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EFFECT OF SOLAR RADIATION ON TRANSPIRATION OF *HELIANTHUS ANNUUS*

EMMETT V. MARTIN

(WITH FIVE FIGURES)

Introduction

Considerable attention has been given to radiant energy as a factor in the rate of transpiration. Although the early investigations were for the most part qualitative in character, all demonstrated the strong accelerating action of radiation. BRIGGS and SHANTZ (4, 5) have made extensive observations of the daily march of transpiration and environmental factors, and have found a high degree of correlation between water loss and radiation. Recently, ARTHUR and STEWART (2) have measured the rate of transpiration of *Nicotiana tabacum* under controlled conditions in the laboratory, and find that to double the radiation increases the rate of water loss approximately 1.7 times. However, an attempt to separate the effect of radiation from that of other environmental factors by means of measurements taken under natural conditions has never been made. From the point of view of the ecologist analyzing the factor complex, such determinations are of considerable value. In an experiment of this sort, it is necessary to change the amount of radiation received by the plants without materially altering the other environmental factors. These conditions were fulfilled in the present work by the use of specially constructed shade tents that transmit different amounts of radiation but allow such freedom of air circulation that the other factors are not appreciably affected.

Methods

In these experiments two types of shade tents were employed. The first consisted of a plane framework of wood 3 feet square covered with cloth, the weave and number of layers determining the intensity of radiation transmitted. These screens were supported at a distance of 1 to 2 feet from the plants and were moved every few minutes during a series so as to maintain the plants in shadow. The second type was somewhat more elaborate in design, as shown in figure 1. The main arch is a 20-foot piece of 3-inch redwood batten bent into the form of a semicircle, while the lower arch is 10 feet long and of similar curvature. The centers of these two pieces are connected by a 4-foot piece of ½-inch iron rod and their ends by 6-foot pieces of batten. This structure is supported with the main arch in an east-west line by five stakes driven in the ground, one at each end of the 6-foot crosspieces and one at the center of the lower arch.

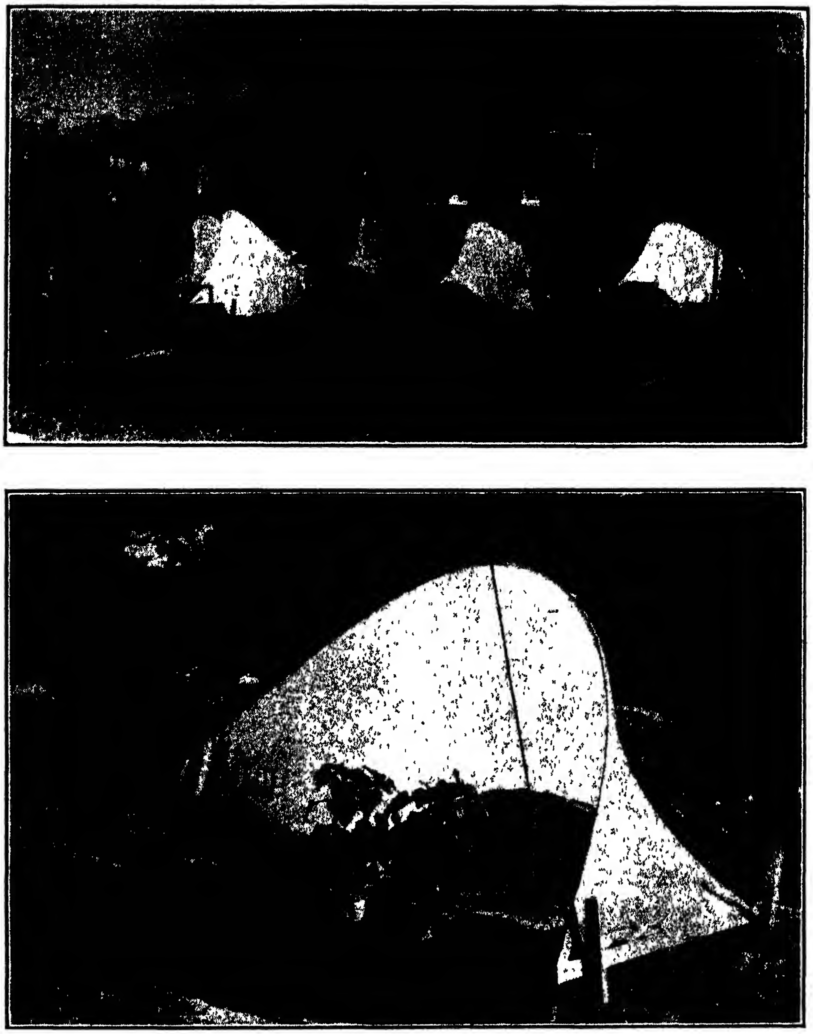

FIG. 1. Above, distant view of entire group of tents; below, close-up view of tent *D*.

Five of these tents were used, each covered with a different type and thickness of cloth as follows: *B* with one layer of cheesecloth with a thread count of 20×16; *C* with one 28×24; *D* with one each of 20×16 and 36×32; *E* with one layer of sheeting 56×60; and *F* with two layers of medium-weight white canvas. The iron rod across the center of each tent was removable, thus permitting the tents to collapse when not in use, a feature that was found to be necessary for even moderately windy days. A wooden box, 4 feet long, 2 feet wide, and 1 foot deep, to hold the phy-

tometers, was sunk in the ground in the center of the space covered by the tent, with its long side in an east-west line. During the fall and winter months, these tents cast the desired shadow all day; but for use in summer, they would need to be constructed differently.

For some of the series, plants of *Helianthus annuus* were grown from seed in water-tight, cylindric, galvanized iron cans 8 inches in diameter and 10 inches in height, while for others similar cans $8\frac{1}{2} \times 11$ inches were employed. Each of these was fitted with a removable lid that had in its center a circular opening 2 inches in diameter. The soil used was a good loam of as nearly uniform texture and moisture as was feasible. The holard was set at approximately 65 per cent. of saturation (or 28 per cent. of the dry weight of the soil), and the total amount of water in each can was kept within 15 per cent. of this value throughout the growing period; and during all series it was maintained constant within 3 per cent. When necessary, water was added through a glass tube extending into a layer of gravel about 1 inch thick in the bottom of the cans. When the plants were a few weeks old, the containers were sealed by adding a layer of sand about $\frac{3}{4}$ inch thick on top of the soil, filling the opening in the lid with non-absorbent cotton, and corking the glass tube. The efficiency of this type of seal was tested by means of controls without plants. Weighings made on a torsion balance to the nearest gram during the time the experiments were in progress showed that such containers seldom lost a detectable amount. This seal has an advantage over the paraffin type in that forced aeration is unnecessary (CLEMENTS and GOLDSMITH **6**).

In all series, six sets of from four to six plants each were used, one set under each tent and a control group in the open, the plants in each set being arranged so as not to shade one another. For the whole-day series the plants were placed under the tents before sunrise and weighed every hour throughout the day. For the short series the plants were put in position about half an hour before the first weighing, which was usually about ten or eleven o'clock in the morning, allowed to remain undisturbed for two to four hours (the time depending upon weather conditions), and again weighed. On account of the fact that clouds cause such rapid fluctuations in radiant energy, series could be run only during periods of cloudless skies. Measurements of air temperature, relative humidity, and average wind velocity were taken every hour during all series. Leaf areas in square decimeters were determined by multiplying the product of the length and width of the leaves in centimeters by the factor 0.0134 (**6**).

Measurements of solar radiation intensity under each tent and in the open were taken two or three times each hour by means of the Smithsonian pyranometer (**1**), which records the intensity of radiation (direct plus diffuse) falling on a horizontal surface. The average intensity during a series

was determined by plotting the observed values on cross-section paper, connecting consecutive points by straight lines as in figure 3, reading the values from this graph for every ten or fifteen minutes, and averaging them.

Results

The transpiration data and radiation measurements for the whole-day series of November 2, 1934, are given in tables I and II respectively, and

TABLE I

TRANSPIRATION DATA OF NOVEMBER 2, 1934

Time		Tent					
		A (control)	B	C	D	E	F
A. M. 7–8	W* =	30	27	47	20	25	23
	t =	0.98	0.97	0.95	0.93	0.90	0.87
	T =	0.27	0.27	0.41	0.19	0.28	0.27
8–9	W =	118	97	105	67	64	54
	t =	0.90	0.92	0.90	0.90	0.90	0.92
	T =	1.14	1.02	0.96	0.86	0.71	0.59
9–10	W =	203	136	154	109	83	65
	t =	1.00	0.98	0.98	0.97	0.98	0.97
	T =	1.77	1.35	1.30	0.99	0.85	0.68
10–11	W =	252	198	205	161	131	103
	t =	1.03	1.05	1.08	1.10	1.08	1.10
	T =	2.12	1.83	1.57	1.30	1.21	0.95
11–12	W =	270	220	229	183	145	115
	t =	1.02	1.02	1.00	1.00	1.00	1.00
	T =	2.30	2.09	1.89	1.62	1.45	1.16
P. M. 12–1	W =	323	253	269	225	177	148
	t =	1.18	1.18	1.18	1.18	1.18	1.18
	T =	2.38	2.08	1.88	1.69	1.50	1.27
1–2	W =	224	189	199	161	128	105
	t =	0.88	0.87	0.85	0.83	0.82	0.82
	T =	2.22	2.11	1.94	1.72	1.56	1.29
2–3	W =	275	233	257	213	174	142
	t =	1.02	1.02	1.03	1.05	1.08	1.12
	T =	2.34	2.22	2.06	1.80	1.61	1.28
3–4	W =	222	179	179	153	110	90
	t =	1.02	1.02	1.02	1.03	1.03	0.98
	T =	1.89	1.70	1.45	1.31	1.07	0.93
4–5	W =	81	60	63	52	42	33
	t =	0.95	0.93	0.95	0.93	0.90	0.93
	T =	0.74	0.63	0.55	0.50	0.47	0.36

* W, water loss in grams per set of five plants; t, time interval between weighings in hours; T, transpiration rate in grams per square decimeter leaf area per hour. The leaf areas in square decimeters for the six sets are: A = 115, B = 103, C = 121, D = 113, E = 100, and F = 99.

TABLE II

MEASUREMENTS OF TOTAL RADIATION INTENSITY ON NOVEMBER 2, 1934*

TIME	TENT					
	A (CONTROL)	B	C	D	E	F
7:00– 7:06	0.032	0.032	0.029	0.029	0.028	0.023
7:57– 8:05	0.413	0.239	0.275	0.080	0.092	0.044
8:29– 8:38	0.557	0.441	0.394	0.216	0.119	0.049
8:50– 8:59	0.652	0.487	0.440	0.251	0.136	0.051
9:24– 9:32	0.77	0.607	0.492	0.315	0.151	0.054
9:52– 9:59	0.83	0.65	0.537	0.314	0.156	0.054
10:22–10:32	0.93	0.73	0.571	0.349	0.167	0.057
10:52–11:00	0.99	0.77	0.66	0.371	0.177	0.061
11:29–11:37	1.02	0.78	0.67	0.372	0.182	0.061
11:52–12:01	1.01	0.74	0.66	0.396	0.172	0.061
12:31–12:38	0.99	0.73	0.60	0.406	0.168	0.061
1:05– 1:14	0.88	0.70	0.548	0.381	0.158	0.061
1:41– 1:48	0.81	0.583	0.504	0.354	0.146	0.061
1:59– 2:05	0.77	0.573	0.480	0.342	0.139	0.058
2:28– 2:37	0.68	0.494	0.405	0.273	0.138	0.051
2:58– 3:07	0.568	0.407	0.355	0.244	0.122	0.044
3:37– 3:43	0.360	0.248	0.204	0.163	0.091	0.039
4:03– 4:08	0.216	0.168	0.126	0.117	0.065	0.032
4:35– 4:41	0.037	0.037	0.031	0.055	0.032	0.021
5:01–	0.008					

* Values of total radiation intensity are in calories per square centimeter per minute.

are represented graphically in figures 2 and 3. In both figures curve A

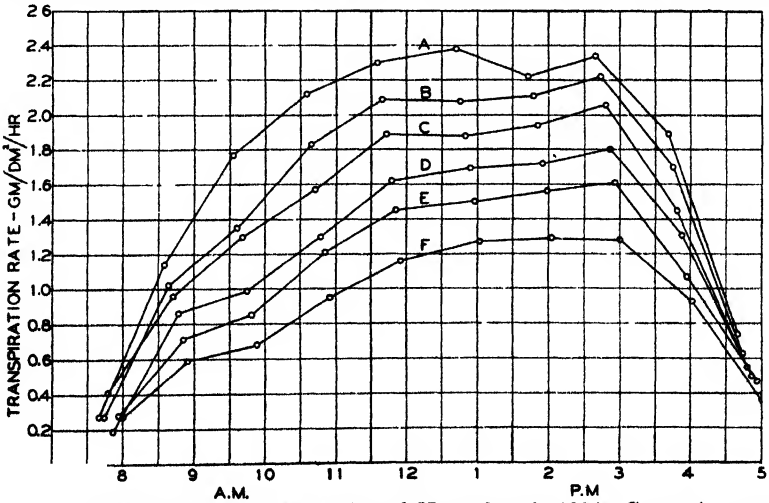

FIG. 2. Transpiration rates for series of November 2, 1934: Curve A corresponds to the open-air control and curves B to F to tents *B* to *F* respectively.

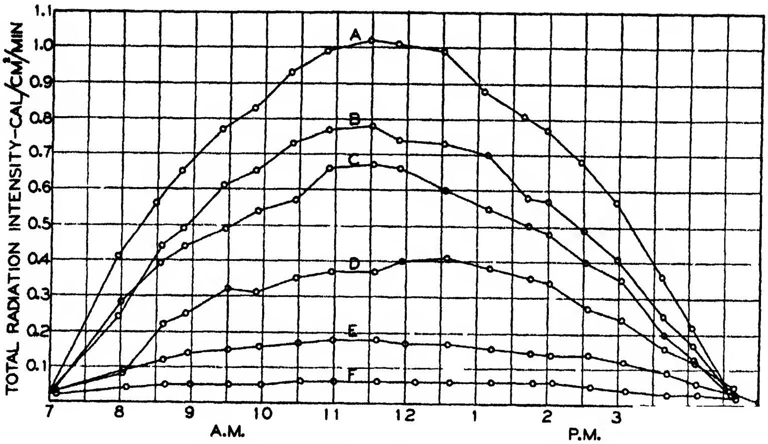

FIG. 3. Total radiation intensities for series of November 2, 1934: Curve A corresponds to the open-air control and curves B to F to tents *B* to *F* respectively.

corresponds to the open-air control and curves B to F to tents *B* to *F* respectively. The transpiration curves for all six conditions are similar in the course of their daily march, but the absolute values decrease with lower radiation. The daily march is controlled by the variations in radiation and meteorological factors throughout the day; but while the radiation differs considerably for the various tents, the other factors are practically identical. Air temperature in the open rarely varied more than 1° from that under tent *F*, as can be seen in table III, while the averages for the two agreed very closely. The construction of the tents being such as to permit fairly free circulation of air, relative humidity and wind velocity

TABLE III

METEOROLOGICAL DATA FOR NOVEMBER 2, 1934

	TIME OF DAY										
	7	8	9	10	11	12	1	2	3	4	5
Air temperature (open)*	50	61	67	68	70	72	74	75	76	75	68
Air temperature (tent *F*)	50	61	67	68	71	73	75	75	75	74	67
Relative humidity	80	58	45	53	40	46	43	40	34	30	46
Wind velocity		0.1	0.1	0.5	3.3	3.8	4.8	3.3	3.8	4.8	0.6

* Air temperatures are in ° F.; relative humidity in percentage; wind velocity in miles per hour. Each value of wind velocity is the average for the hour preceding the time of day for which it is given.

TABLE IV

RADIATION AND TRANSPIRATION DATA FOR SHORT SERIES

Date	Time limits		Tent					
			A (control)	B	C	D	E	F
4/21/33	10:00–2:30	R* =	1.36	1.04	0.81	0.576	0.343	0.144
		T =	1.76	1.43	1.18	1.01	0.66	0.55
		r_T =	± 0.035	± 0.060	± 0.034	± 0.049	± 0.040	± 0.020
5/13/33	12:15–3:00	R =	1.32	1.00	0.82	0.550	0.355	0.143
		T =	1.66	1.29	1.21	1.07	0.93	0.66
		r_T =	± 0.044	± 0.038	± 0.032	± 0.041	± 0.020	± 0.031
3/27/34	10:45–3:00	R =	1.09	0.74	0.61	0.454	0.257	0.130
		T =	2.21	1.69	1.58	1.38	1.17	0.93
		r_T =	± 0.032	± 0.029	± 0.038	± 0.021	± 0.022	± 0.020
3/31/34	10:30–3:00	R =	1.26	0.87	0.75	0.475	0.273	0.082
		T =	2.74	2.41	2.32	1.92	1.63	1.34
		r_T =	± 0.061	± 0.062	± 0.071	± 0.046	± 0.059	± 0.035
8/1/34	9:15–11:00	R =	1.45	1.15	0.96	0.75	0.529	0.058
		T =	2.12	1.95	1.85	1.61	1.36	0.99
		r_T =	± 0.076	± 0.037	± 0.036	± 0.045	± 0.021	± 0.034
8/9/34	10:30–1:30	R =	1.40	1.13	0.79	0.494	0.205	0.061
		T =	4.90	4.70	4.45	3.92	3.30	3.14
		r_T =	± 0.112	± 0.132	± 0.107	± 0.026	± 0.133	± 0.098
8/11/34	10:00–11:45	R =	1.39	1.06	0.71	0.415	0.208	0.086
		T =	3.86	3.61	3.01	2.75	2.69	2.58
		r_T =	± 0.031	± 0.175	± 0.108	± 0.021	± 0.058	± 0.078
10/11/34	10:15–3:15	R =	0.94	0.72	0.570	0.374	0.198	0.088
		T =	2.75	2.28	2.09	1.90	1.62	1.33
		r_T =	± 0.045	± 0.028	± 0.043	± 0.031	± 0.039	± 0.032
10/22/34	11:00–3:15	R =	0.93	0.63	0.592	0.348	0.157	0.059
		T =	2.23	1.77	1.85	1.40	1.16	1.08
		r_T =	± 0.115	± 0.045	± 0.055	± 0.042	± 0.036	± 0.051
11/2/34	11:00–3:15	R =	0.87	0.65	0.526	0.345	0.150	0.055
		T =	2.31	2.13	1.94	1.71	1.53	1.25
		r_T =	± 0.040	± 0.009	± 0.032	± 0.051	± 0.033	± 0.037

* R, average radiation intensity in calories per square centimeter per minute for period between weighings; T, average transpiration rate in grams per square decimeter per hour for same period; r_T, probable error in T calculated from values given by the various individuals in each set of plants (number of plants in each set is shown in table V).

likewise showed very little difference, so that only the values of these factors obtained from measurements in the open are given in the table.

The transpiration and radiation data for the short series are recorded in table IV, while meteorological and other data are included in table V. The series of August 1 was run at Windy Point, elevation 12,000 feet, on the slopes of Pike's Peak; those of August 9 and 11 at Colorado Springs, Colorado, elevation 6000 feet; and all others at Santa Barbara, California, practically at sea level. The results from five of these series are shown graphically in figure 4, which portrays transpiration rate as a function of

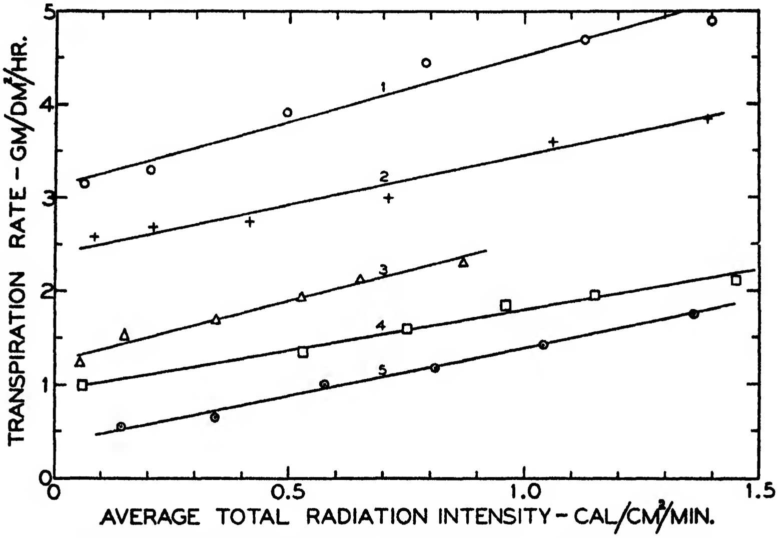

FIG. 4. Results of short series of April 21, 1933 (5), August 1, 1934 (4), August 9, 1934 (1), August 11, 1934 (2), and November 2, 1934 (3).

radiation intensity. Curve 1 represents the data of August 9, curve 2 those of August 11, curve 3 those of November 2, curve 4 those of August 1, and curve 5 those of April 21. In all cases, not only does the rate of transpiration increase with higher intensity of radiation, but the rate of increase is linear. Accordingly the data from all short series are best represented graphically by straight lines, or mathematically by equations of the type $T = b + mR$, where T is the rate of transpiration, R the radiation intensity, b the intercept on the T axis, and m the slope of the straight line. From the point of view of the plant, b should represent the transpiration rate as determined by the combined action of all factors other than radiation, while m should be a measure of the direct effect of the latter.

The method of least squares (various texts, and BIRGE **3**) was employed in obtaining from the various series the values of b and m and their probable errors (table V). Both m and b were found to vary considerably for

the different series. Since b is influenced by several factors, it does not show a high degree of correlation with any particular one. In general, however, it increases with higher evaporating power of the air; m, on the other hand, does not vary in the same manner as b. This is readily discernible from table V, but is also clearly shown by the data from the whole-day series of November 2. If the average transpiration rates and radiation intensities for the period 8–9 are used, the value of b is found to be 0.406 ± 0.012 and that of m is 1.203 ± 0.037. Similarly, the data for the period 12–1 yield $b = 1.200 \pm 0.027$ and $m = 1.194 \pm 0.046$. The former is distinctly different for the two periods, while the latter remains constant; hence m is apparently not affected by changes in environmental factors such as take place during the day. On this basis the observed changes in m for different days must be ascribed to some factor or factors in which changes from day to day take place. Two outstanding examples of these are size and age of plants. There appears to be no correlation between average leaf area and m, but there does seem to be a definite relation with age, which is graphically shown in figure 5. Both m and the rate of change of m with age decrease with increasing age. Of the ten points plotted,

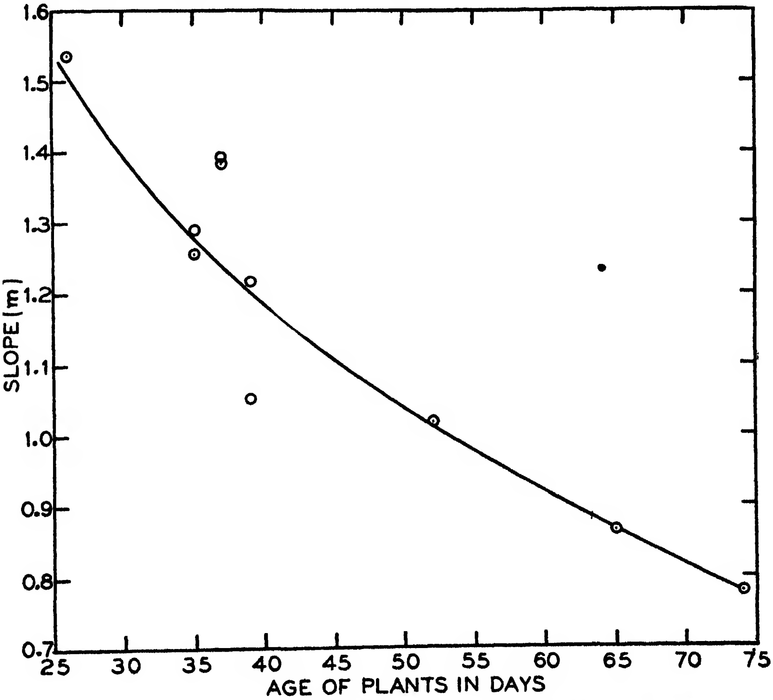

FIG. 5. Slope of transpiration rate *vs.* radiation intensity curves as a function of age of plants in days.

only two are off the curve by more than their probable errors, and these by only twice that amount.

An indication of the importance of radiant energy as a factor in transpiration is furnished by the ratio $mR/(mR+b)$, which gives the fraction of the rate of water loss that is ascribable to the direct effect of radiation. Since this ratio is influenced by a number of factors, it varies over a wide range of values and is usually larger on cold humid days than on warm dry ones. Under the conditions in these experiments, it varied from 38 to 81 per cent., but under more extreme conditions a greater range could be expected.

Leaf temperatures of the plants in the open and under tent *F* were taken at noon for three of the series by means of the well known thermocouple method (**7**). The temperature of leaves hanging nearly at right angles to the sun's rays in the open averaged about 1° C. above that of the air, while that of leaves under tent *F* was about 5° C. below. These temperatures, however, fluctuated so much with air currents and for different leaves on the same plant that no attempt was made to obtain the average for entire plants under the various conditions.

Accuracy of results

The designers of the pyranometer (**1**) claim that it is accurate to 1 per cent. However, the method used in the present work to obtain the average over a period of a few hours introduces another factor of uncertainty, which can only be estimated; but it seems conservative enough to place the probable error in the average intensities given in table IV at from 2 to 3 per cent.

Errors in the measurements of the rate of transpiration may be grouped into two classes, accidental and constant or systematic. The former includes errors in weighing, in measurements of leaf areas, and those caused by structural differences in the plants themselves. Since these will appear in the results as variations among the individuals in each set of plants, an estimation of their magnitude is furnished by the probable errors in the values of T. These were calculated by the method of least squares from the individual values that were averaged to obtain T and are given in table IV as r_T. Expressed in percentage of T, these range from 0.4 to 6.1 with an average of 2.7 per cent.

An estimation of the accuracy with which the data are represented by equations of the type $T = b + mR$ was obtained from the deviations of the observed values of T from those calculated from these equations with the appropriate values of m and b. Expressed in percentage of the calculated values, these deviations range from 0 to 8.3 with an average of 3.0 per cent. Since these are of the same order of magnitude as the probable errors in T, the relation between rate of transpiration and intensity of radiation can

be considered linear within the limits of experimental error. These deviations are responsible for uncertainty in the values of b and m, given in table V with their probable errors which in the case of b range from 2.2 to 6.2 with an average of 3.5 per cent., and in the case of m from 2.8 to 7.0 with an average of 4.8 per cent.

The second class of errors (constant or systematic) includes those caused by such factors as stomatal opening, time required to weigh the entire group of plants, and differences in the quality of radiation under the various tents. The degree of opening of the stomata of each set of plants was determined at hourly intervals during the whole-day series and most of the short ones by means of the alcohol method (**10**), but at no time was any appreciable difference found. The uncertainty introduced by the time taken to weigh the entire group of plants (20 to 25 minutes) has been discussed in an earlier paper (**7**) and shown to be negligible under the conditions of these experiments.

Ivanov and Thielman (**9**) have shown that the rate of transpiration of potted plants may be 50–60 per cent. greater under blue-violet than under red-yellow light of the same intensity. Accordingly this factor must be considered as a possible source of error in these investigations, since there is a gradual shift in the wave length of maximum intensity in passing from open air condition *A* to tent *F*. The plants in the open receive full sunlight, while those under tent *F* get only the light reflected from nearby objects and that from the north sky with its greater proportion of short wave lengths. The operation of this factor would tend to increase the transpiration rates in the lower range of intensity, which would appear in the results in the form of larger values of b and smaller values of m than would be obtained with no differences in quality.

Discussion

Leclerc du Sablon (**11**) has suggested that the accelerating effect of radiation on transpiration may be due partly to heating of the leaves and partly to a change in the permeability of the protoplasm. Confirmation of the second part of this hypothesis has been furnished by Ivanov and Thielman (**9**), who have shown that the transpiration of potted plants may be 50–60 per cent. greater under blue-violet light than under red-yellow light of the same intensity. They suggest that this effect is due to a greater influence of the blue-violet on the permeability of the protoplasm. Lepeschkin (**12**) has shown that the permeability of the protoplasm of *Elodea* leaves to methylene blue increases with increase of light up to 10 per cent. of full sunlight, but for intensities above this there is very little, if any, change. Since practically all the intensities used in the present work are above this value, it may be that change of permeability plays a

TABLE V

ADDITIONAL DATA FOR SHORT SERIES

DATE	AV. AIR TEMP.	AV. REL. HUMIDITY	AV. WIND VELOCITY	AIR PRESSURE	AGE OF PLANTS	AV. LEAF AREA PER PLANT	TYPE OF TENT	PLANTS PER SET	b*	m*
	°F.	%	m.p.h.	cm. Hg	days	$dm.^2$				
4/21/33	66	51	1.8	76	52	11.9	Sq.	4	0.372 ± 0.023	1.018 ± 0.029
5/13/33	64	53	2.6	76	74	14.1	Sq.	4	0.595 ± 0.034	0.779 ± 0.043
3/27/34	71	62	1.9	76	35	13.2	Arc	6	0.808 ± 0.023	1.255 ± 0.036
3/31/34	74	41	1.7	76	39	18.6	Arc	6	1.306 ± 0.042	1.216 ± 0.058
8/ 1/34	62	41	2.0	49	65	6.0	Sq.	4	0.937 ± 0.034	0.865 ± 0.036
8/ 9/34	86	31	7.6	61	37	5.7	Sq.	4	3.123 ± 0.081	1.393 ± 0.097
8/11/34	82	41	1.8	61	39	6.8	Sq.	4	2.402 ± 0.053	1.051 ± 0.067
10/11/34	76	53	1.6	76	26	6.8	Arc	6	1.254 ± 0.041	1.534 ± 0.073
10/22/34	73	48	1.6	76	37	25.3	Arc	4	0.956 ± 0.029	1.383 ± 0.054
11/ 2/34	73	41	3.9	76	35	21.7	Arc	5	1.254 ± 0.034	1.289 ± 0.066

* Values of b and m given are accompanied by their probable errors calculated by the method of least squares.

minor rôle in the results, and that the accelerating action of radiation observed is due largely to its heating effect. No estimate of the relative magnitude of the effects of these two factors was obtained in these experiments, since it would require knowledge of air and leaf temperature differences as well as of the amount of radiant energy absorbed by the leaves, both of which are much too variable under natural conditions to be measured accurately. The fact that the relation between radiation and transpiration is linear, however, indicates that any change of permeability that may exist under the conditions in these tests will be either small in comparison to the heating effect or directly proportional to the intensity of radiation.

Similarly, the cause or causes of the variation of the accelerating effect of radiation with age of the plants (fig. 5) cannot be determined from the results of these investigations. Since the slope of the straight lines (m) represents the direct effect of radiation on the transpiration rate, the decrease that occurs in it with increasing age of the plants must be associated with changes in the influence of radiation. If the latter is of a twofold nature, as already mentioned, these changes in m may be brought about in either of two ways. The changes that take place in the leaves as they grow older may be such that the coefficient of absorption for radiation is reduced so that the heating effect is less pronounced, or they may be such that the influence of radiation on the permeability of the protoplasm becomes less effective with increasing age.

Summary

1. The influence of radiation on the rate of transpiration of *Helianthus annuus* has been measured under natural conditions by the use of shade tents constructed so as to transmit different amounts of radiation and to permit such freedom of air circulation that other environmental factors were not altered appreciably.

2. The ten series made covered a rather wide range of conditions, but in all cases the relation between radiation intensity and transpiration rate was found to be linear within the limits of experimental error.

3. The results of all series could be satisfactorily represented by equations of the type $T = b + mR$, where T is the transpiration rate, R the radiation intensity, b the intercept on the T axis, and m the slope of the straight line.

4. It was found that b varied directly with the evaporating power of the air and m inversely with the age of the plants.

5. The fraction of the transpiration rate due to the direct effect of radiation is given by the ratio $mR/(mR + b)$, and for plants in full sunlight was found to vary from 38 to 81 per cent., depending largely upon the evaporating power of the air.

6. Calculations of probable errors indicate that on the average the results are accurate to within 5 per cent.

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METHOD FOR QUICKLY DETERMINING NITROGEN IN PLANTS, AND SOLUBLE NITROGEN AS A MEASURE OF THE NITROGEN AVAILABLE FOR ANABOLIC PROCESSES[1]

E. M. Emmert

Part I: Determination of total nitrogen

In previous papers (1) a method was described for determining total nitrogen by oxidizing with sodium chlorate and sulphuric acid, distilling and determining nitric acid in the distillate by the phenoldisulphonic acid method. This procedure, although more rapid than the standard Kjeldahl method, requires considerable time and equipment, and the technique is rather exacting. Recent work has shown that, by regulating conditions properly, distillation can be dispensed with and the desired result accomplished by adding phenoldisulphonic acid directly to the mixture after oxidation.

Procedure

Put 100 to 300 mg. of finely ground, well averaged, dry tissue or 500–1500 mg. of green tissue into a 200-cc. Erlenmeyer flask. There should be at least 0.5 mg. of nitrogen in the sample used. Add 1 gm. of sodium chlorate for each 100 mg. of dry sample or each 500 mg. of green sample. Add 25 cc. of 50 per cent. by volume sulphuric acid and attach the flask to a water-cooled reflux condenser. Heat with a high flame until the oxidation is complete and all chlorine color has disappeared from the solution (usually 3 to 5 minutes). Remove the flame, flush out the condenser with 10–15 cc. of 50 per cent. sulphuric acid, cool somewhat (a cold water bath may be used), detach from the condenser and make the solution to 50 cc. with 50 per cent. sulphuric acid. Immediately put exactly 1 or 2 cc. of the solution into a suitable graduated flask, add 3 or 6 cc. of phenoldisulphonic acid, mix well, add about 20 cc. of water, make alkaline with 40 per cent. NaOH solution, adding it until the maximum yellow color is produced and make to volume. After thorough mixing, the clear solution is compared with a standard in a colorimeter. The volume of the solution should be such that the color is fairly close to that of the standard.

Notes

1. In low nitrogen samples larger aliquots may be used, but the phenoldisulphonic acid should be increased in proportion. It may be advan-

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

tageous to keep the amount of acid lower so that the dilution can be less than 50 cc., although too little acid should not be used since the efficiency of the oxidation may be reduced.

2. If the solution after phenoldisulphonic acid addition is yellow, the decomposition of chloric acid was not complete and the heating was not continued long enough.

3. Most of the chlorine is expelled as chlorine gas, but some chloride is left in the acid solution. When the aliquot was small and the phenoldisulphonic acid was added to the 50 per cent. acid solution immediately after the oxidation instead of to dry salts, no loss of nitrate could be detected. A standard nitrate solution was treated with chlorate as described in the procedure and no loss was detected, even in larger aliquots, when the phenoldisulphonic acid was added to the 50 per cent. acid solution. The presence of a small amount of water to keep the salts in solution overcomes the loss caused by chloride, which would be experienced if phenoldisulphonic acid were applied to dry salts. Evidently nitrosyl chloride is not formed in the presence of water, and as long as the acid is not diluted too much the reaction between nitrate and phenoldisulphonic acid is complete.

4. Presence of much iron, manganese, or perhaps some other heavy metals causes loss of nitrate nitrogen. Error from this source is not appreciable in the analysis of normal plant tissues since the amount of heavy metals in relation to the nitrogen present is very small. In soils and some fertilizers, however, enough heavy metal may be present to cause loss. The writer has devised a procedure for soils (5) in which the metals are precipitated in the presence of excess oxidizing agent, before adding phenoldisulphonic acid.

5. The reaction with chlorate usually proceeds smoothly without the formation of chlorine dioxide. If green fumes of chlorine dioxide should start accumulating it is usually because too low a flame was used and the air in the flask above the solution was not raised to over 100° before the solution got hot. Even then, with the small amount of chlorate used, chlorine dioxide seldom accumulates enough to cause a serious explosion.

6. A round-bottom flask should not be used since the reaction is more likely to be violent. The wide bottom of an Erlenmeyer spreads out the solution and does not allow action to be concentrated at one point. Reflux of water usually washes back any particles of sample which stick to the sides of the flask. If a high flame is used the solution boils up enough to reach most particles on the sides of the flask.

7. On cooling, sodium sulphate may crystallize out of the neutralized yellow solution, especially if the volume is small. For accurate work it is

best not to remove these crystals, since the hydration of the crystallized salts tends to concentrate the solution and cause a high result. It is best to warm the solution a little or keep it warm enough to prevent crystallization. In fact, the most accurate colorimetric readings are obtained immediately after dilution to the final volume, before the solution has a chance to change temperature very much and cause slight volume changes or crystallization of salts.

Results

Tables I and II show the results obtained on some plant tissues of different kinds. The Kjeldahl results were obtained by the chemistry, agronomy, and feed control departments of the Kentucky Experiment Station in their regular routine work. These results were taken as correct

TABLE I

Total nitrogen found by the Kjeldahl and chlorate methods (percentage of dry tissue)

Sample	Kjeldahl method	Chlorate method	Percentage error
	%	%	%
Tobacco			
Leaves (cured)	2.85	2.84	−0.35
Young plants	4.35	4.35	0
Medium aged plants	3.96	3.98	+0.51
Old plants	3.55	3.51	−1.13
Roots	1.48	1.42	−4.05
Lespedeza	3.38	3.40	+0.58
Egg mash (1)	3.07	3.065	−0.16
Egg mash (2)	3.14	3.13	−0.32
Purina calf chow	4.25	4.31	+1.41
Dairy ration	3.23	3.13	−3.09
Mixed feed	2.23	2.38	+6.72
Cottonseed meal	6.26	6.25	−0.16

TABLE II

Tomato tissue (percentage of green tissue by chlorate method)

Sample	Determination 1	Determination 2	Percentage error from average
	%	%	%
Plant 1, stems	0.1137	0.1126	0.44
Plant 2, stems	0.1042	0.1000	2.01
Plant 1, leaves	0.3677	0.3572	1.52
Plant 2, leaves	0.4167	0.4167	0
Young lettuce plants	0.2273	0.2232	0.88

in figuring the percentage of error. Table II presents duplicate results obtained on green tissue samples.

These results indicate that the presence of water does not affect the determination. In fact it is to be expected that water would not interfere, since 50 per cent. sulphuric is used. The interference by water is a serious objection to the Kjeldahl method when used on certain samples, as shown by RANKER (**6**).

The errors from theoretical are not large excepting in the case of pyridine (table III). The stable sulphate formed with pyridine evidently

TABLE III

DETERMINATION OF NITROGEN IN PURE ORGANIC COMPOUNDS

COMPOUND	THEORETICAL N ADDED	N FOUND	PERCENTAGE ERROR
	mg.	*mg.*	%
Picric acid .	18.337	18.48	+ 0.77
Picric acid ..	9.168	9.20	+ 0.34
Sulphanilic acid	6.692	6.67	− 0.33
Sulphanilic acid	6.692	6.54	− 2.27
Strychnine sulphate	3.27	3.26	− .33
Pyridine . . .	8.849	2.20	− 75.25

was attacked very little by the oxidizing agent. It will be noted, however, that strychnine gave almost theoretical results. Evidently alkaloids which are derivatives of pyridine or quinoline are readily oxidized if side chains are present, but when no side chain is present to form a path into the ring, the alkaloid is not broken down. The Kjeldahl method is not effective on pyridine or quinoline either.

The low results with sulphanilic acid were probably due to slight impurities since this acid is difficult to obtain absolutely pure.

The nitrogen in a mixture of 50 mg. of pyridine and 50 mg. of young tobacco plant (same sample as in table I) was determined in the usual way. The nitrogen found gave exactly 4.35 per cent. nitrogen for the tobacco, leaving no N for pyridine. Evidently the pyridine was not attacked at all in the presence of the tobacco.

Part II: Determination of soluble nitrogen

In 1929 a method for the rapid determination of nitrate nitrogen was published (**3**). This method has proved valuable in work with succulent plants, especially of the stem tissues which are rather easy to free of interfering organic substances. The use of copper hydroxide produces satisfactory extracts for the application of the phenoldisulphonic acid method, where the amount of nitrate nitrogen is high in relation to the carbo-

hydrate present, as in succulent, rapidly growing stem tissues of tomatoes, lettuce, cabbage, etc. In leaf tissues and in woody stems, however, interference by carbohydrates and other organic compounds is often encountered. It was found that legumes with zero nitrate grew luxuriantly. Evidently these plants were getting all the nitrogen for growth in a form other than nitrate. There is reason to believe that considerable nitrogen is absorbed from the soil and soil air in forms other than nitrate, especially through the action of root nodules and by fertilization with ammonium sulphate or calcium cyanamid. TIEDJENS and ROBBINS (7) and others have shown that ammonia nitrogen is freely assimilated by plants. In order to overcome interference by soluble organic compounds and also to determine the total nitrogen available for metabolism, a method for determining the total nitrogen in plant extracts was devised. This nitrogen, since it includes all nitrogen in true solution in the plant extract, was termed soluble nitrogen. The method consists in oxidizing all material completely by the use of sodium chlorate. This causes all the nitrogen to be converted to nitrate and decomposes all organic compounds, leaving only nitrate and traces of metals, phosphate phosphorus, etc., in solution. The interference by organic material is entirely overcome. Most of the chlorine is driven off; some chloride is present after oxidation, but this does not cause loss of nitrogen as previously explained in note 3 under the total nitrogen determination. Notes 2, 4, and 7 also apply here.

PROCEDURE

Introduce by means of a graduated pipette 0.1–0.5 cc. of the plant extract depending on the concentration of nitrogen (extraction with weak acetic acid as previously described (4) seems preferable), into the bottom of a test tube (20 × 150 mm.) without wetting the sides of the tube. The sample should contain at least 0.005 mg. of nitrogen. The addition of reagents should be in proportion to the size of the aliquot used. Add a granule of sodium chlorate about the size of a grain of wheat. Add 0.2–1.0 cc. of fuming sulphuric acid (15 per cent. SO_3) rather rapidly and without shaking so that the solution will boil and excess chloric acid will be decomposed. Shake and blow across the top of the tube until all the chlorine is expelled as indicated by the disappearance of the greenish yellow color and the formation of a perfectly clear, colorless solution. Immediately add 0.1–0.5 cc. of phenoldisulphonic acid and shake. After about 30 seconds add 2.0–10 cc. of water and clear 40 per cent. sodium hydroxide until the maximum yellow color develops and an excess of alkali is present. Care must be taken, if a large aliquot and amount of reagent have been used, to prevent excessive boiling and loss of solution. It may be necessary to transfer to a larger vessel for the neutralization. Make the yellow solution to a volume which brings the color fairly close to the color of the standard

used and compare in a colorimeter. The standard used for results presented here contained 0.0025 mg. of nitrogen per cc. A 1- or 2-gm. sample of green tissue was extracted by grinding with about 0.1 gm. acid-treated charcoal and 10 cc. of 2 per cent. acetic acid.[2]

Variations in soluble nitrogen

Table IV shows that the method gave very good results on aliquots from the same sample, and that the differences between samples from the same

TABLE IV

Variations in amounts of soluble nitrogen between aliquots of same extract, petioles of same plant, and plants within a few feet of each other on same field plot

Plant	Petiole	Aliquot 1 p.p.m.	Aliquot 2 p.p.m.	Percentage of general average
				%
1	1	696	696	96.4
	2	696	690	96.0
	3	580	580	80.3
Average		656.3		90.9
2	1	696	696	96.4
	2	617	617	85.4
Average		656.5		90.9
3	1	867	867	120.1
	2	906	906	125.5
Average		886.5		122.8
General average		722.1		100.

plant probably were due to actual variations in soluble nitrogen in the different petioles taken for analysis. It is possible that the variations were at least partly caused by variations in the extraction process, but it is natural to expect fluctuations in petioles taken from different parts of the plant and which must vary to some extent in age and size. Of course, an even larger variation is to be expected between different plants even if they presumably are in the same soil type.

Comparisons between soluble and nitrate nitrogen

The question naturally rises as to how much more soluble nitrogen there is than nitrate nitrogen. Table V shows that plants differ widely in the ratio of soluble to nitrate nitrogen although the ratio was quite constant for a particular type of plant in most cases. Lima beans gave an indefinite

[2] Weakened fuming sulphuric acid or isolation of particles of chlorate on the sides of the tube may result in incomplete decomposition of chloric acid. This will act on phenoldisulphonic acid and cause off-color tints.

TABLE V

COMPARISON OF AMOUNTS OF SOLUBLE NITROGEN TO NITRATE NITROGEN IN SAME PLANT EXTRACT. EFFECT ON PHOSPHATE PHOSPHORUS IN THE EXTRACT (P.P.M. OF GREEN TISSUE)

SAMPLE	SOIL AND TREATMENT IN LB. PER ACRE	SOLUBLE N	NITRATE N	RATIO SOLUBLE N: NITRATE N	PHOSPHATE P
		p.p.m.	*p.p.m.*		*p.p.m.*
Tomato (young) lower petioles	Red clay	4167	910	4.6	320
Tomato (young) lower petioles	Red clay	4310	910	4.7	266
Tomato (young) lower petioles	Red clay and superphosphate, 500 lb.	3012	1000	3.0	364
Tomato (young) lower petioles	Red clay and superphosphate, 500 lb.	3049	1062	3.0	......
Tomato (old) lower petioles	Black loam	2500	625	4.0	645
Tomato (old) lower petioles	Black loam	2777	625	4.4	645
Tomato (old) lower petioles	Black loam	2777	555	5.0	667
Cabbage (half-grown) midribs	Red clay plus $(NH_4)_2SO_4$, 250 lb.	1661	1190	1.4	80
Cabbage (half-grown) midribs	Red clay plus $CaCN_2$, 250 lb.	805	570	1.4	100
Cabbage (half-grown) midribs	Red clay plus $NaNO_3$, 250 lb.	1340	925	1.4	74
Cabbage (half-grown) midribs	Check	670	570	1.2	143
Peaches growing tips	Black loam	260	55	4.7	222
Peaches growing tips	Black loam plus $(NH_4)_2SO_4$, 3 lb. per tree	416	0	∞	92
Lima beans (old plants) main stems	Red clay (check)	250	0	∞	25
Lima beans (old plants) main stems	Red clay plus superphosphate, 2000 lb.	261	0	∞	83
Lima beans (old plants) main stems	Red clay plus 8 ton limestone	160	0	∞	39
Lima beans (old plants) main stems	Black loam (check)	420	0.	∞	125
Lima beans (old plants) main stems	Black loam plus nitrate, 400 lb.	2500	1390	1.8	31

ratio since the nitrate was zero. They evidently were feeding entirely on nitrogen other than nitrate. However, when large amounts of nitrate were added considerable nitrate was present in the stems and caused a ratio of 1.8. Tomatoes maintained a ratio between 3.0 and 5.0, while cabbage was a still more vigorous collector of nitrate, maintaining a ratio of 1.4 even in the presence of ammonium sulphate and calcium cyanamid. The data on peaches indicate that peaches may collect nitrogen in the ammonium form if so supplied.

Effect of soluble nitrogen on phosphate phosphorus

Except on soil to which superphosphate was added, increase in soluble nitrogen in the plant was almost always associated with a marked decrease in phosphate phosphorus. This is brought out very strikingly in table V. Evidently, when plenty of soluble nitrogen is present, phosphate phosphorus becomes limited because of the stimulus to rapid growth and consequent rapid utilization of the phosphate supply.

Relation of soluble nitrogen and phosphate phosphorus to plant vigor and yield

Increases in soluble nitrogen correlate with plant vigor but not always with yield. This is shown in table VI, by the lima beans. Medium sized vigorous bean vines on level highland, with a medium soluble nitrogen and relatively high phosphate content, yielded six times as much as large, dark green, vigorous vines in a rich lowland. The content of soluble nitrogen in those in the lowland was three times as large, and that of phosphate was larger than in those on the highland. Despite the higher phosphate, the yield was smaller because the very high soluble nitrogen threw the vines to vegetative growth. In beans the soluble nitrogen-phosphate ratio is one of the most important factors determining yield. Determinations of nitrate in lima beans would not mean much, especially if vigorous nodule formation takes place. The same would probably hold true with peaches as with lima beans. It is especially hard to determine nitrate in peaches since organic substances (probably largely amygdalin) interfere in the test.

Main stems of lima beans, large new shoots of peaches (not the growing tip), and large midribs of cabbage leaves were used for analysis (table VI). Different shoots from the same tree were used in securing the duplicate results on peaches. The yields of cabbage (variety Jersey Wakefield) are pounds for 200 feet of row. The season was rather dry and the heads small.

In cabbage, however, the determination of nitrate is easy and probably is significant, although soluble nitrogen is undoubtedly about as significant. It seems, however, that cabbage is an especially heavy feeder of nitrate and

TABLE VI

RELATION OF SOLUBLE NITROGEN AND PHOSPHATE PHOSPHORUS TO PLANT VIGOR AND YIELD

Crop	Location or treatment	Soluble N	Phosphate P	Ratio N/P	Notes on vigor and yield
Lima beans	On sloping land with considerable wash	143	95	1.5	Comparative yields at one picking, 8 lb. (green beans and pods) per 200 ft. of row
	On level high land	298	160	1.9	Per 200 ft. of row, 25 lb.; medium sized vigorous green vines
	In rich bottom	893	190	4.7	Per 200 ft. of row, 4 lb.; very large over-vegetative dark green vines
Peaches	On top of hill (medium slope)	699	571	1.2	Leaves yellowish and dropping off; trees medium size (samples taken July 13)
		767	571	1.2	
	At bottom of hill	1736	1111	1.6	Vigorous large trees with dark green leaves, very few leaves dropping
		1838	900	2.0	
Cabbage	Check	670	143	4.7	Yield 58 lb.; light green and small
	250 lb. $CaCN_2$ per acre	805	100	8.1	Yield 84 lb.; dark green and large
	250 lb. $NaNO_3$	1340	74	18.1	Yield 113 lb.; dark green and large
	250 lb. $(NH_4)_2SO_4$	1661	80	20.8	Yield 71 lb.; medium green and fairly large

does best when fertilizer is added as nitrate, since sodium nitrate produced the largest yield, despite the fact that ammonium sulphate produced the highest soluble nitrogen content. Previous work (2) indicates that an acid soil reaction lowers utilization of nitrogen in the meristem while an alkaline reaction favors its utilization. Since ammonium sulphate tends to produce acidity and sodium nitrate, alkalinity, this may explain why ammonium sulphate caused an accumulation of soluble nitrogen in the cabbage midrib, while sodium nitrate produced larger yields despite the slightly lower soluble nitrogen reserve in the midribs. Anything which slows up nitrogen

utilization is likely to cause an accumulation of soluble nitrogen in the conducting tissues if there is a vigorous nitrogen uptake by the roots. The fact that calcium cyanamide produced a larger yield than ammonium sulphate, despite a lower soluble nitrogen content, gives further proof of the beneficial effect of alkaline fertilizers on cabbage, since cyanamide is much more alkaline than sodium nitrate and probably causes a rapid nitrogen utilization. Tiedjens and Robbins (7) show definitely that ammonia is assimilated as ammonia in an alkaline reaction in sand cultures and that the tomato and soy bean make better growth with ammonium hydroxide than with either nitrate, calcium, or ammonium sulphate. Poor growth was made with ammonium sulphate except when the pH was around 8. Peach, apple, and rose seedlings grew best with ammonia in an alkaline medium. Nitrate was assimilated best in an acid reaction, however, although growth was not so good as with ammonia in an alkaline reaction.

It seems from the data presented in this paper and from previous work on tomatoes, that an extensive study to determine the optimum amounts and ratios of soluble nitrogen and phosphate phosphorus for each type of plant would be of great technical interest and practical importance.

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DEVELOPMENT OF THE CHLOROPHYLL AND CAROTENOID PIGMENTS IN BARLEY SEEDLINGS[1]

G. Mackinney

Introduction

This paper reports the results of some experiments showing the effect of modification of the supply of iron and potassium on the formation of the plastid pigments in barley seedlings. In many types of experiments dealing with vital phenomena it is virtually impossible to segregate direct from indirect effects. In the present case, one method would be to show that if other vital processes have not been stimulated so much as pigment formation, then the effect of the element under consideration has probably been direct, and it has a rôle in the mechanism of pigment formation. The difficulty is that the simplest measure of vital activity, increase in dry weight, is itself dependent, although in no simple way, on the chlorophyll itself. While all vital processes may be affected by a particular treatment, the significance of changes in the pigment content is not necessarily vitiated by corresponding growth changes. The writer has attempted a different technique. By withholding iron, plants were allowed to become chlorotic while growing in solutions ample and restricted with respect to potassium. Chlorophyll formation (and therefore growth to some extent) hinged on iron as the limiting factor. Iron was subsequently added to determine whether the previous nutritional treatment had affected pigment formation. In this paper, however, the emphasis will be placed on certain observed pigment interrelationships the ratios of which are unaffected by growth considerations, since it is realized that without extensive studies of potassium nutrition on growth, ranging from definite deficiencies to luxury consumption of this element, with resultant changes in pigment content, this aspect of the question cannot be satisfactorily settled.

Methods of analysis

The transmission (T) and thence the optical densities (D) of extracted solutions of the various pigments were determined in a spectral region of high absorption for each pigment with a Bausch and Lomb spectrophotometer. The method has been described by McNicholas (2) and Smith (9).

The angle θ' of rotation of the prism, for a match point, was determined in one field (average of ten readings) and the corresponding θ'' with reversed fields.

Whence $\log T = D = \log \cot \theta' + \log \tan \theta'' = Ech$, where c is the concen-

[1] Contribution from the Division of Plant Nutrition, University of California.

tration of the solution, h the height of the column, and E a constant dependent on the wave length and the nature of the substance.

To obtain E, solutions of known concentration of the pure pigment were prepared and their optical densities determined. At different dilutions, with columns of different height, the constant E could be determined with an accuracy of ± 2 per cent. By means of a vernier, the plunger could be set to 0.1 mm. By using open cups with plungers instead of sealed tubes, and the less volatile solvents, rapid adjustment of the height of the column was possible. In this way intensities at the match point were adjusted for minimum visual strain and greatest sensitivity. The standards were prepared essentially by the method of SCHERTZ (6). As several leaf carotenes are optically inactive, the carotenoids were prepared from sunflower leaves. Carrot root carotene is undesirable as it contains 10–20 per cent. of the alpha component.

In unknown solutions, chlorophyll was determined directly in an aliquot of the crude extract. The carotene and xanthophyll were then isolated from the remainder and determined separately, to avoid interference. Comparisons were made for chlorophyll in acetone solution, at 665 mμ; for carotene, in acetone at 500 mμ; and for xanthophyll, in 90 per cent. methanol at 485 mμ.

Experimental errors

As noted, the constant E was determined with an accuracy of ± 2 per cent. Plant variability was minimized by rigid selection of those seedlings which at the end of a specified time made uniform growth response to constant environmental conditions. Taking the xanthophyll pigment, which showed the greatest variation, in experiment 1, the mean of the xanthophyll in triplicate determinations involving thirty plants is 1.08 mg. ± 0.04 per ten plants. We may estimate therefore the gross experimental error to be not greater than ± 5 per cent. It must be pointed out that when an element such as iron is present in limiting amounts, control sets do not represent duplicates since minute traces of iron both in the plant and as impurity in the solution will affect the pigment content. Equally rigid exclusion of iron is impossible in cultures of this type, nor is this necessary beyond the point where the plant becomes sensitive to the low concentration of iron.

Experimentation

With variations in the pigment content under various treatments there will be variations in respirable carbohydrates, and consequently in absorption of minerals from the culture solution. In certain experiments these differences have been minimized by subjecting the plant to a given treatment for a period of time, then transferring it to dilute calcium sulphate

solution for observation. In so far as pigment formation is determined by mineral nutrition, changes can occur only to the extent that the plant has stored up the necessary minerals. This necessitates experiments of relatively short duration.

Experiment I: Pigment ratios in normal and chlorotic barley seedlings

Barley seedlings of height 6 cm. ± 0.5, seven days after germination at 20° C., were transferred to twenty 1-quart jars (six plants per jar) containing Hoagland's solution, with supplementary constituents[2] except for iron. Ten of these jars received 5 mg. Fe from a 1 per cent. solution of ferrous sulphate per week. Iron was excluded from the remainder. Plants from cultures with and without iron were harvested periodically in duplicate sets. The results are shown in table I. Agreement is to be anticipated for

TABLE I

Pigments in normal and chlorotic barley plants (mg. per 10 plants)

	Chlorophyll	Carotene	$Q \frac{a+b}{c}$	Xanthophyll	$Q \frac{c}{x}$
	mg.	*mg.*		*mg.*	
Initial	1.03	0.0336	30.7	0.0948	0.35
	Normal series (iron added)				
8 days	6.54	0.222	29.5	0.508	0.44
10 days (1)	9.70	0.32	30.3	0.594	0.54
(2)	9.38	0.312	30.0	0.474	0.66
Outer leaf	{ 2.62	0.085	30.8	0.118	0.72
remainder	{ 7.18	0.254	28.3	0.428	0.59
Total (3)	9.80	0.339	28.8	0.546	0.62
12 days	12.82	0.412	31.1	0.744	0.55
19 days	21.4	0.712	30.0	1.15	0.62
	Chlorotic series (iron restricted)				
8 days	4.72	0.16	29.5	0.470	0.34
10 days (1)	3.12	0.110	28.4	0.346	0.32
(2)	4.06	0.156	26.1	0.370	0.42
Outer leaf	{ 2.22	0.076	29.2	0.136	0.56
remainder	{ 3.26	0.122	26.7	0.324	0.38
Total (3)	5.48	0.198	27.7	0.460	0.43
12 days (1)	3.08	0.100	30.8	0.238	0.42
(2)	4.16	0.134	31.0	0.294	0.46
19 days	2.76	0.079	34.7	0.216	0.37

duplicate sets receiving iron; but, as already noted, each set of chlorotic plants will not have had equally rigid exclusions of this element, causing greater variation. In the outside leaves of plants without iron the color was apparently normal, whereas the inside leaves were severely chlorotic.

[2] A–Z constituents, *cf.* Schropp and Scharrer (7).

After a 10-day period, from three jars of each series the outside leaves were sampled for comparison with those inside. The results of the experiment show:

1. The chlorophyll: carotene ratio, Q(a + b)/c, is remarkably constant. One infers a straight line relationship of the type y = mx between the two pigments, in barley seedlings, whether they are developing normally or becoming chlorotic. The relationship between chlorophyll and carotene has been observed by EULER (1) and SJØBERG (8) in etiolated barley seedlings exposed to light, and more generally between chlorophyll and total carotenoids by OSERKOWSKY (5) in normal and chlorotic pear leaves.

2. The carotene: xanthophyll ratio, Q c/x, with normally developing seedlings rapidly approaches the value of about 0.60, determined by WILLSTÄTTER. The Q c/x for chlorotic plants is consistently lower, with one interesting exception, the green outside leaves of plants that are becoming chlorotic.

EXPERIMENT II: PIGMENT FORMATION AS AFFECTED BY HIGH AND LOW CONCENTRATIONS OF POTASSIUM IN THE EXTERNAL SOLUTION

The potassium ion is readily absorbable, and modification of its concentration causes marked effects on the accumulation of Ca^{++}, Mg^{++}, and $(NO_3)^-$ ions. Analyses are given in table II showing that the presence or absence of iron has little effect on potassium withdrawals by the plant. However, nothing is known of the effect of potassium on the absorption of supplementary constituents, such as boron or copper, which are toxic in all but high dilutions although essential for normal growth. It may not be justifiable to assume that the amount of copper required by the plant is so small that a tenfold variation in the K^+ concentration does not, under some conditions, inhibit to a limiting degree the absorption of copper. In experiments II and III, a modified Hoagland solution was used, high K^+ series receiving 200 mg. and low K^+ series 20 mg. of potassium per liter. The latter received the corresponding sodium salts to maintain the total salt concentration.

TABLE II

ABSORPTION OF POTASSIUM

SERIES	K IN SOLUTION		ABSORBED BY PLANT
	INITIAL	FINAL	
1. High K	*mg.*	*mg.*	*mg.*
(a) + Fe	200	72.2	127.8
(b) − Fe	200	67.7	132.3
2. Low K			
(a) + Fe	20	1.5	18.5
(b) − Fe	20	1.0	19.0

Seeds were germinated and selected as before. Thirty-six quart jars were set out, eighteen receiving the high K^+ solution and eighteen the low K^+.

Six of each series were given 5 mg. Fe, as before. The plants were allowed to grow for 10 days in their respective culture solutions. Samples of each treatment from both series were then taken in duplicate. The

TABLE III

PIGMENT CONTENT AS AFFECTED BY IRON AND POTASSIUM MODIFICATIONS (MG. PIGMENT IN 10 PLANTS)

	CHLOROPHYLL	CAROTENE	$Q\frac{a+b}{c}$	XANTHOPHYLL	$Q\frac{c}{x}$
	mg.	*mg.*		*mg.*	
Initial	0.740	0.024	30.8	0.054	0.44
A*-1	6.28	0.160	39.3	0.330	0.48
		0.126		0.257	0.49
B-1	5.92	0.163	36.3	0.278	0.59
	5.52	0.166	33.3	0.316	0.53
A-2	3.80				
	3.00	0.096	31.3	0.307	0.31
B-2	4.18	0.104	40.1	0.248	0.42
	3.63	0.100	36.3	0.210	0.48
	Plants were here transferred to $CaSO_4$ solution: After 4-day period				
A-1	8.07	0.237	34.1	0.467	0.51
B-1	5.12	0.142	36.1	0.288	0.49
A-2a	6.63	0.162	40.9	0.327	0.50
	6.48	0.151	42.9	0.327	0.46
B-2a	4.75	0.131	36.3	0.223	0.59
	4.55	0.129	35.3	0.220	0.59
A-2	3.98	0.106	37.5	0.242	0.44
B-2	3.42	0.090	38.0	0.176	0.51
	After 7-day period				
A-2a	6.24	0.188	33.2	0.391	0.48
	6.03	0.192	31.4	0.342	0.56
B-2a	4.05	0.123	32.9	0.218	0.56
	4.23			0.281	

* A denotes high K; B, low K solutions.
1 " 5 mg. Fe from beginning and 5 mg. at transference to $CaSO_4$ solution.
2 " no Fe.
2a " Fe from time of transference to the $CaSO_4$ solution.

solutions in the remaining jars were then discarded and replaced by dilute calcium sulphate solution. Each series which had not received iron was subdivided: half of them were given 5 mg. Fe and iron was again withheld from the remainder. This was done to determine whether the plants, in recovering from the chlorotic conditions which had developed, would show pigment differences ascribable to their previous nutritional treatment. The

plants were allowed to develop in the dilute calcium sulphate solution, and were sampled in duplicate after four and seven days. No further samples were taken as the plants began to show the effect of restrictions in the nutrient solutions. In table III are given the pigment analyses on the basis of ten plants. It may be noted that, except for color, the plant tops showed no visible differences in development, although roots were whiter and possibly less sturdy in the absence of iron. As already pointed out, however, there is no appreciable difference in the respective potassium withdrawals, so that one may assume that there is no marked difference in the capacities of the root systems for absorption of mineral nutrients.

After the 10-day period in the original solution, the plants without iron have become definitely chlorotic, the high K^+ series approximately as severely as the low K^+. Iron has therefore been the limiting factor in chlorophyll formation, and potassium has not been able to act as a substitute. After the plants have been transferred to the calcium sulphate solution for four days, the chlorophyll content of plants in the high K^+ series is nearly doubled, where iron has been added, while there is less than a 20 per cent. increase in the corresponding plants of the low K^+ series. Where iron has been withheld, no pigment increase can be noted in either series. It will be seen that while there is a fairly definite relationship between the chlorophyll and the carotene, the magnitude of $Q(a+b)/c$ is not strictly comparable with that of experiment I, which was confined to two phases: (1) normal development in seedlings, and (2) arrested development due to incipient iron chlorosis. In this experiment the leaves are recovering from chlorosis under varying nutritional conditions. The ratio $Q\,c/x$ shows a less marked lowering for the low K^+ series than for the high K^+. The general fluctuation of chlorophyll with the carotenoids is noticeable throughout.

Experiment III: Effect of variation in potassium content on pigment formation in the dark

Trays of barley seedlings were germinated at 20° C. in the dark over the solutions high and low with respect to the K^+ concentration. The condition of the plants after 12 days is shown in table IV. Duplicate samples of fifty plants each from both series were taken after 6, 9, 11, and 15 days. Results of pigment analyses are shown in table V. No chlorophyll was detectable. An apparent trace was measurable spectrophotometrically at 665 mμ. But when comparisons were made also at 670 mμ and 660 mμ, the same divergence (less than 0.5°) was noticeable in the setting of the prisms for a match point. Obviously no definite absorption band of chlorophyll was demonstrable. It is possibly not to be expected that seedlings steadily losing weight in the dark should show, in their pigment content, definite

TABLE IV

COMPOSITION OF PLANTS

SERIES	DRY WEIGHT OF 50 PLANTS	K ON DRY WEIGHT BASIS
	gm.	%
High K	1.42	2.44
		2.37 Average 2.405
Low K	1.66	0.47
		0.61 Average 0.54

TABLE V

PIGMENT IN PLANTS GROWN IN THE DARK (MG. PER 50 PLANTS)

	XANTHOPHYLL		CAROTENE		$Q\frac{c}{x}$
	HIGH K	LOW K	LOW K	HIGH K	
	mg.	mg.	mg.	mg.	
6 days (1)	0.046	0.034	0.003	0.002	
(2)	0.080	0.054	0.005	0.005	
Average	0.054		0.004		0.07
9 days (1)	0.111	0.130	0.011	0.020	
(2)	0.102	0.120	0.008	0.014	
Average	0.115		0.013		0.11
11 days (1)	0.107	0.148	0.008	0.015	
(2)	0.173	0.122	0.008	0.017	
Average	0.137		0.014		0.10
15 days (1)	0.156	0.144	0.017	0.017	
(2)	0.178	0.174	0.021	0.020	
Average	0.163		0.019		0.12

response to the solutions. The low values of Q c/x confirm the findings of EULER (1) and SJØBERG (8).

An apparent conflict may be noted between the results of these two workers. The former notes that where carotene is present, it is accompanied by chlorophyll, and its production is ascribed to incomplete darkness. The latter invariably finds carotene, and rarely chlorophyll. Superficially, the writer's results substantiate the latter viewpoint. But conclusions that light may not be necessary for carotene formation in photosynthetic tissue, or that chlorophyll is not necessary for carotene production, are by no means valid. Vital processes in the etiolated seedling have been seriously interfered with. Reactions proceeding in the normal plant have been profoundly modified, and the direction of some may have been reversed. In this paper the writer shows the interdependence of chlorophyll and carotene under certain conditions. This is not true for all conditions. Thus, in green tissue placed in the dark the ratio Q(a + b)/c falls, while Q c/x rises. Therefore the reactions which have brought about traces of carotenoid pigments in the etiolated seedlings may not be those which

are responsible for pigment production in the illuminated plant. In fact there is no evidence as yet of the chemical identity of either carotenoid occurring in the etiolated plant with those of normal green tissue. To obtain production of xanthophyll for example, one must not interfere with the processes which produce chlorophyll. The only valid statement permissible is that the present technique and equipment permitted the measurement of traces of carotene where chlorophyll was not present in measurable amounts.

Summary

1. Fluctuations are reported in the chlorophyll and carotenoid contents of barley seedlings grown for short periods of time in culture solutions modified with respect to iron and potassium.

2. The constancy of the chlorophyll: carotene ratio noted by EULER and HELLSTRÖM for etiolated seedlings is extended to cover chlorotic seedlings and also those recovering from chlorosis induced by lack of iron.

3. Chlorophyll and carotenoids fluctuate together, in general, although the carotene: xanthophyll ratio is markedly lower in chlorotic leaves.

4 The effect of potassium, whether specific or one of general nutrition, under certain conditions is to increase the pigment content of the plants.

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ADDENDUM.—In the light of recent developments in methods, a few comments appear pertinent, to attempt to assay more accurately the value of the data presented.

In this work, the greatest fluctuation occurred in the xanthophyll fraction. It has been noted by STRAIN (10) for a number of plants, including barley, that the leaf xanthophyll fraction contains several components, while β carotene is probably the sole constituent of the carotene fraction of this plant. It is evident therefore that a measure of uncertainty is introduced, as any assumption that there has been no change in the proportions of the various components of "total" xanthophyll may be justified only under a limited set of conditions.

Exposing etiolated sunflower seedlings, germinated at 85° F., to light, NORRIS (4) finds a close relationship between chlorophyll and xanthophyll, but none between these pigments and carotene, contrary to the work of EULER and HELLSTRÖM (1) with etiolated barley seedlings. NORRIS utilized a spectrophotoelectric method, isolating the 4358 Å. mercury line for analyses of the two carotenoid fractions. In the experience of MILLER, MACKINNEY, and ZSCHEILE (3) the absorption curves for various carotenoids show greatest reproducibility where the curves are flatter, at the maxima.

For visual work, unless one is matching the intensities of two semicircles in a circular field, it is impracticable to use narrow exit slits. With the Bausch and Lomb spectrometer no. 2700 the writer found it convenient to isolate a spectral region of approximately 6 mμ in magnitude, for chlorophyll (*i.e.*, 668–662 mμ) and from 2.5 to 3.0 mμ for the other pigments. These were not varied for standards and unknowns. Subsequent observations on crude carotenoid extracts, by visual and spectrophotoelectric methods, and to a limited extent by photographic means, lead the writer to believe that while the positions of maxima and minima are identical on the wave-length scale, within the experimental error, with those of the crystalline carotenoid in the same solvent, the gen-

eral shape of the curve is different, the bands being more diffuse and less clearly defined. This might be ascribed to unavoidable traces of a colored and partially oxidized form of the carotenoid.

If this observation be confirmed, there are obvious inherent errors which may affect absolute though not comparative results for methods dependent on a single "wave length" for the determination, especially if that wave length is not in a region of maximum absorption for the pure pigment.

As pointed out, the chlorophyll: carotene relationship found for one set of conditions does not exist for all conditions. The divergent results in the literature emphasize the necessity of critical examination of the method to be used, and the urgency of preliminary studies, by adsorption methods, of the carotenoid complexes which may exist in the plant chosen for study, before satisfactory conclusions can be drawn as to the functions of these pigments in the living leaf.

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ABSORPTION SPECTRA OF ALPHA AND BETA CAROTENES AND LYCOPENE[1]

Elmer S. Miller, G. Mackinney,[2] and F. P. Zscheile, Jr.

(WITH THREE FIGURES)

This paper presents a detailed study of the specific absorption coefficients of alpha and beta carotenes purified by resolution on magnesia, according to the method of Strain (5), and on calcium hydroxide according to the method of Miller (3), and of lycopene. The absorption coefficients were measured by an improved spectro-photoelectric method which will be described in due course.

From the viewpoint of carotenoid spectroscopy, several important changes have been made. The period required for a single reading has been reduced from 60 to 10 seconds. The length of the liquid path in the absorption cells is 2 cm. ± 0.15 per cent. The Hilger (type D, no. H291) absorption cells have been placed behind slit number II of the monochromator, where they are not subjected to the full intensity of the illuminating source, in this case, a 900-watt Mazda lamp.

In order that comparisons with values obtained in other laboratories may be valid, we deem it essential to state: (1) slit widths in mm. actually employed at the various wave lengths, and (2) the effective dispersion, *i.e.*, the slit width in terms of Å. U. per mm. of slit. From these data the spectral range isolated may be calculated. These calibration data of the optical system must be obtained from constants determined by means of a steady source of monochromatic light of suitable wave lengths. A mercury arc was used in this case.

In this paper are reported values of the specific absorption coefficients for alpha and beta carotenes and lycopene in the wave length range 3900–5300 Å. U. The solvent was 20 per cent. diethyl ether and 80 per cent. absolute ethanol (by volume). 1.5–3.0-mg. samples were weighed on a Kuhlmann microbalance, with a maximum error of approximately ± 0.3 per cent. The samples were dissolved in ether and made up to volume with alcohol. Concentrations employed were approximately 0.6–1.0 mg. per liter. Using fused quartz cells with these concentrations, the light transmission at the maxima for alpha and beta carotenes was from 20–40 per cent. On different preparations, from various sources, values of α[3] at the

[1] Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

[2] National Research Council Fellow. This investigation employed apparatus made available by a grant from the Rockefeller Foundation, New York, to the University of Chicago.

[3] From the formula $\log \frac{I_x}{I_o} = \alpha\, c\, x$, where α is in liters per gram cm.

maxima (and between 4350 and 4800 Å. U.) were reproducible within ± 0.6 per cent. (total error). For the steeper portions of the curves (on either side of the spectral range between 4350–4800 Å. U.) the total error is ± 1.0 per cent. Absorption coefficients on different samples of the same preparation show that the magnitude of the instrumental and weighing errors contribute to the total error approximately one-half that stated above.

The following slit widths were employed: 0.04–0.01 mm. for wave lengths between 3900 and 4300 Å. U., and 0.01–0.007 mm. between 4300 and 5300

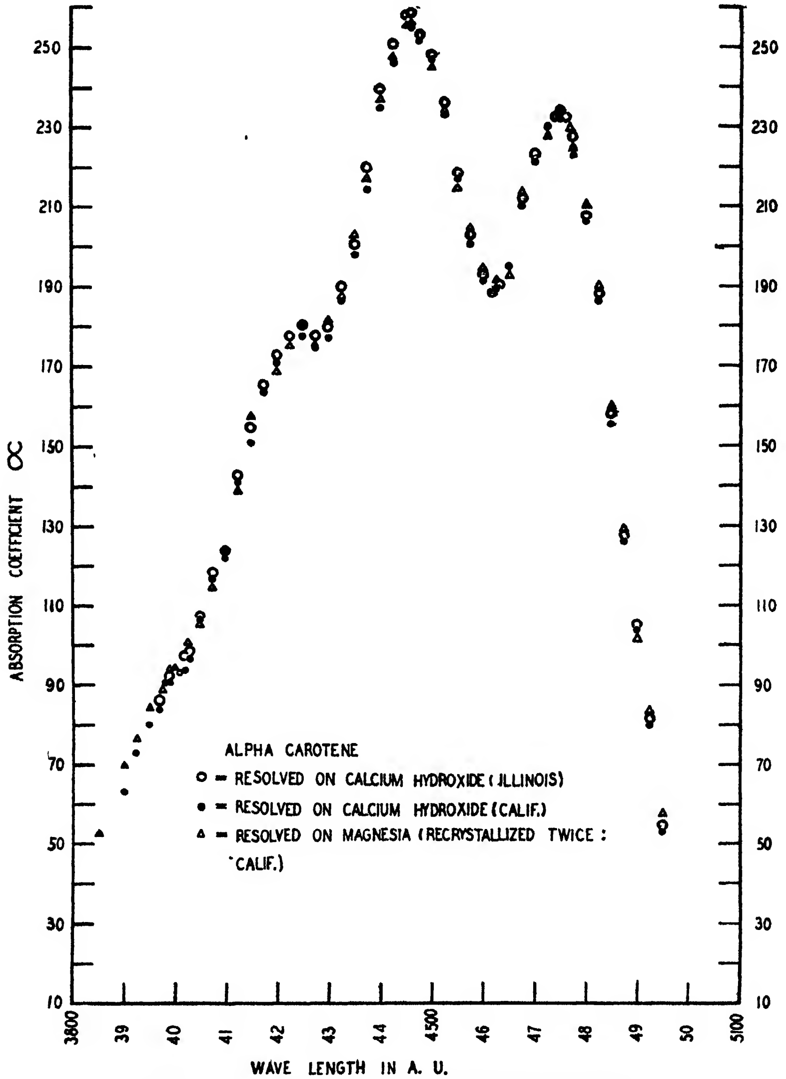

FIG. 1. Absorption spectrum of alpha carotene.

Å. U. These slit widths in terms of Å. U. are 2.5–1.0 and 1.0–1.5 Å. U. respectively. The spectral regions isolated at wave lengths 3900, 4300, and 5300 Å. U. were 7.5, 3.0, and 4.5 Å. U. respectively. When these slit widths were employed, no change was observed in the value of the specific absorption coefficients when a mercury arc was substituted for the Mazda lamp. (Beta carotene and the mercury lines at 3906, 4047, 4078, 4358, and 4916 Å. U. were employed). In order that workers in different laboratories may

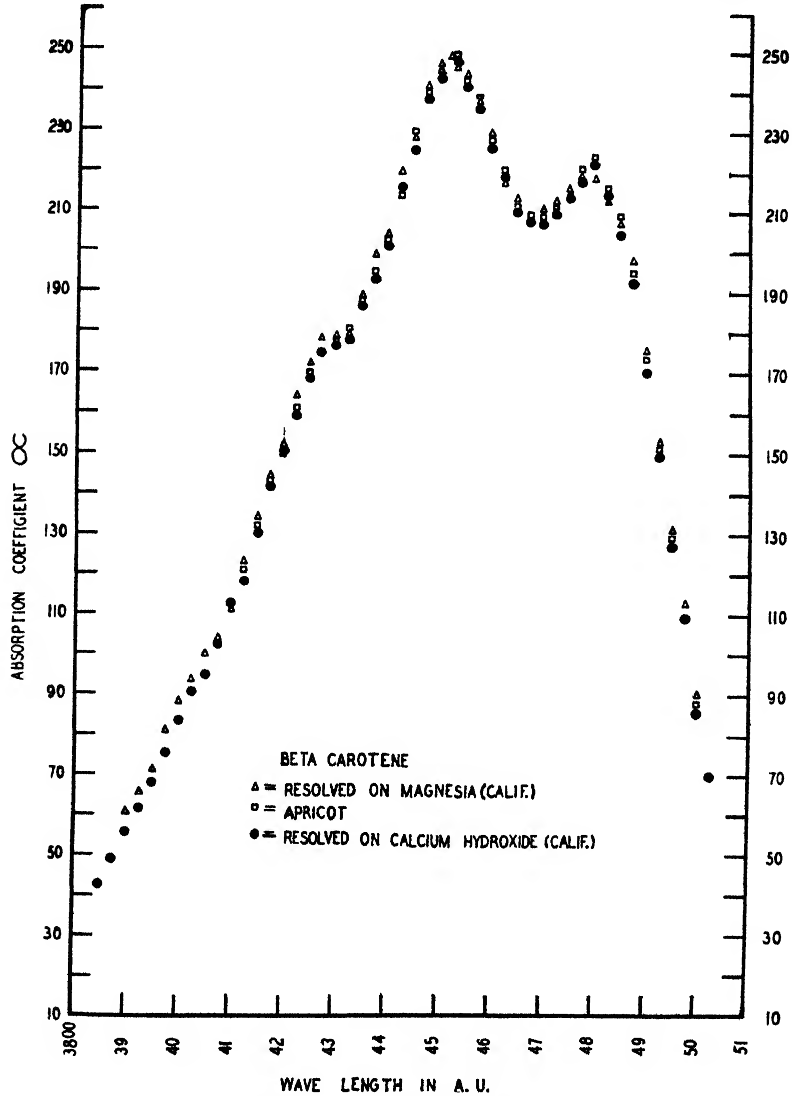

FIG. 2. Absorption spectrum of beta carotene.

compare values, it is essential that data concerning slit widths in terms of mm. and Å. U. per mm., and the spectral limits of the light employed be included with the values of the absorption coefficients.

Experimentation

Preparations and purification of alpha and beta carotenes

The sources of the crude alpha and beta carotenes were carrot roots grown in California and Illinois. The carotenes were purified by the methods of Strain (5) and Miller (3). After elution from the respective adsorbents, all the preparations were recrystallized from ligroin[4] rich in heptane and dried for 10 to 14 hours at $2\text{–}40 \times 10^{-5}$ mm. pressure. The samples were recrystallized until a constant set of values for the absorption coefficients was obtained.

Absorption spectra of alpha and beta carotenes

Readings were taken at 10 Å. U. intervals over the maxima and minima, and at 25 Å. U. intervals for the remaining portions of the curve. The maxima and minima can be located within $\pm$ 5 Å. U. In figure 1 is shown the absorption spectrum of alpha carotene, consisting of 3 bands and a shelf. Maxima for the two main bands occur at 4750 Å. U. ($\alpha = 231.5 \pm 1.5$), and at 4460 Å. U. ($\alpha = 257 \pm 1.5$), with a minimum at 4620 Å. U. ($\alpha = 191.0 \pm 1$). A third band is found at approximately 4250 Å. U. ($\alpha = 178 \pm 2$). While we consistently get a dip in the curve at 4260–4270 Å. U., the differences in this region at three wave lengths 10 Å. U. apart are approximately that of the experimental error. The shelf occurs at 4010 Å. U.

In the curve for beta carotene (fig. 2) are found two definite maxima, at 4800 Å. U. ($\alpha = 222 \pm 1.2$) and at 4525 Å. U. ($\alpha = 249 \pm 1.5$), with a minimum at 4700 Å. U. ($\alpha = 210 \pm 1.2$). A distinct shelf occurs at 4300 Å. U. and an apparent point of inflection at 4050 Å. U.

The values of the absorption coefficients are approximately 15 per cent. higher for alpha carotene, and 4 per cent. higher for beta carotene than those reported by Miller (4) showing that previous results are low, though in shape the two sets of curves are almost identical. This difference may in part be ascribed to a slit width approximately one-quarter the width of those previously used, and in part to better absorption cells. However, these two factors cannot account for the lower value of alpha carotene in comparison with beta carotene.

The curves presented in figures 1 and 2 show the identity of alpha and beta carotenes isolated from carrot roots from California and Illinois whether separated on magnesia or on calcium hydroxide. Preparations of

[4] Using ligroin (B.P. 30–60° C.) it was found desirable to add 10 per cent. heptane to give satisfactory resolution.

beta carotene from the leaves of mint, dandelion, and apricot, isolated by MACKINNEY (1), gave on recrystallization curves identical with those reported in figure 2. A comparison of the curves (figs. 1 and 2) shows the values of the specific absorption coefficients for alpha carotene to be slightly higher than those for beta carotene at their respective maxima.

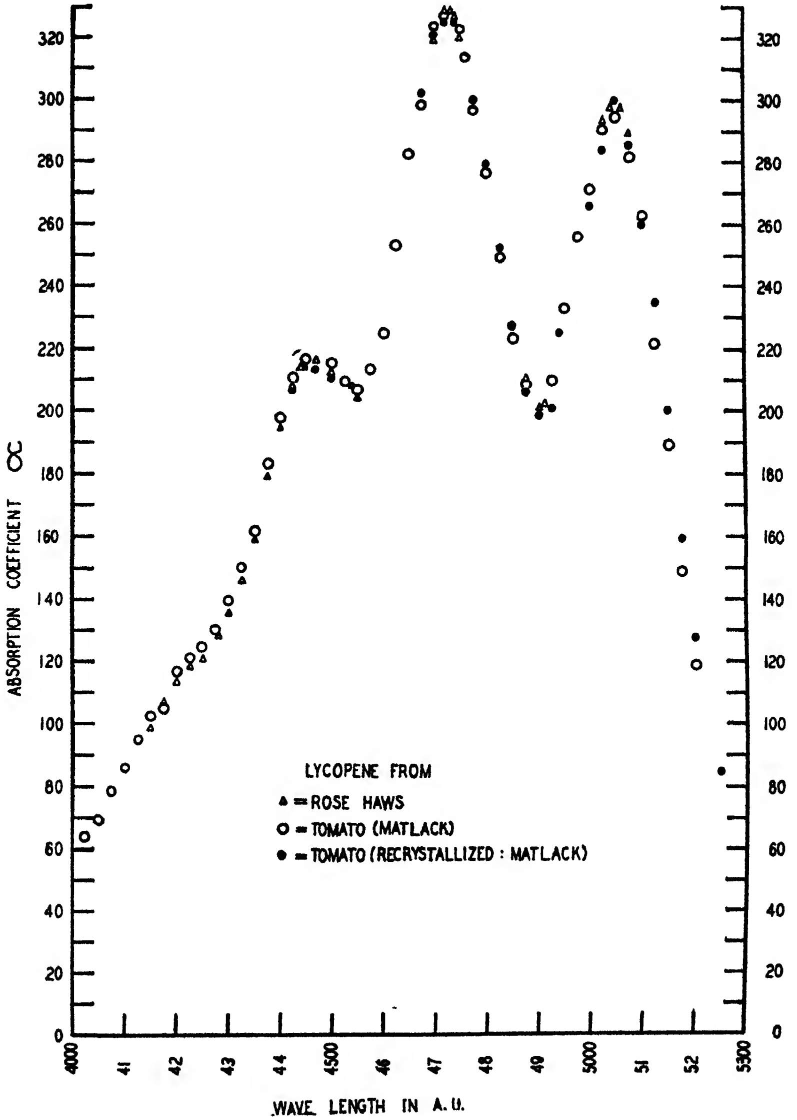

FIG. 3. Absorption spectrum of lycopene.

Lycopene

Lycopene was obtained from two sources, one isolated by Mackinney (1) from the hips of the California wild rose, the other from tomatoes, furnished by courtesy of M. B. Matlack (2). The rose hips lycopene was isolated from accompanying carotenoids by passage of the petroleum ether crude extract over magnesia according to the method of Strain (5). A check was thus provided on the possible effect of the adsorbent on this pigment.

Absorption spectrum of lycopene

Three well defined maxima were found at 5050 ($\alpha = 298 \pm 2$), 4725 ($\alpha = 327 \pm 2$), and 4465 ($\alpha = 217 \pm 1$) Å. U. Minima were found at 4900 ($\alpha = 201 \pm 2$) and at 4550 ($\alpha = 204 \pm 2$) Å. U. An apparent point of inflection was noted between 4200 and 4250 Å. U. The values for the absorption coefficients at the maxima were determined with the same degree of precision as that described for alpha and beta carotene.

A less rigorous examination was made of the other portions of the curve. Owing to their steepness, we estimate an uncertainty of $\pm$ 2.0 per cent.

Within the relatively narrow concentrations employed here (0.6–1.0 mg. per liter) we have not observed significant deviations from Beer's law. For the most accurate work, between 20 and 40 per cent. of the incident light must be transmitted.

Summary

Curves of the specific absorption coefficients for alpha and beta carotene and lycopene are presented for the wave length range from 3900 to 5300 Å. U. The carotenes from two different localities resolved into their components on two different adsorbents, magnesia and calcium hydroxide, yield identical products. A discussion is presented of the desirability, in all future work, of stating the magnitude of error and certain fundamental details of the optical system employed, particularly with regard to the purity of the spectral region isolated at the various wave lengths where measurements are made.

Acknowledgment is made of the helpful suggestions of Dr. T. R. Hogness, Department of Chemistry, University of Chicago, and of the cordial cooperation of members of the Division of Plant Biology, Carnegie Institution of Washington.

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CHEMICAL ANALYSIS OF PLANT TISSUE[1]

The present observations and recommendations supplement those previously issued by this committee (**3**). Since that period the subject has received increasing attention by investigators, as becomes apparent in the character of numerous published articles. Appreciative acknowledgment is here made of aid received from a number of persons in the development of this supplement.

It is noteworthy that an extensive treatise for use in research has appeared recently under the direction of Klein (**9**). Increasing aid is also found in laboratory manuals of biochemistry, as for example the work of Cole (**2**).

Sampling.—In the case of fruits the number of individuals required for a representative sample received attention some time ago by Denny (**4a**). He found with apples, for example, that 50 units give significance to a difference of 5 per cent. in acidity. The necessity for determination of absolute amounts of a given constituent per plant unit in following its appearance or disappearance with metabolic changes has been demonstrated by Kertesz (**8a**). By the treatment of peas in groups of equal numbers he demonstrated the fallacy of a common belief that sucrose is converted to starch when canning of the harvested crop is delayed.

Preservation.—In view of information recently made available it appears desirable to qualify the application of preservative treatments to either tissue or extracts. It may thus be possible to aid the individual worker in the selection of a method adapted to his conditions.

A. Alcoholic extraction.—Apparently extracts obtained by the use of boiling 80 per cent. alcohol are stable but a few days at room temperatures, at least as regards some of the nitrogenous constituents. Webster (**13**) has reported the occurrence of appreciable deaminization soon after cooling such extracts. It is recommended that changes of composition in stored extracts be retarded by holding at low temperatures, preferably below 0° C.

B. Freezing.—Enzyme action is not prevented by the freezing of plant tissues. On the contrary, ruptures caused by this treatment seem to bring enzymes and substrates into contact. For this reason, heat treatment should precede freezing when the samples are to be stored for more than a few weeks. Frozen samples should be wrapped or sealed air tight to prevent dehydration from evaporation, which proceeds rapidly in frozen tissue at − 18° C. The temperature of the storage room should be at most − 18° C.,

[1] First supplement to Recommendations of the Committee on Methods of Analysis for the American Society of Plant Physiologists. This report was assembled by W. E. Tottingham for the committee on chemical methods: Z. I. Kertesz, W. E. Loomis, T. G. Phillips, W. E. Tottingham, chairman.

but it is preferable to operate at $-40°$, where as yet enzyme action has not been shown to have measurable velocity. On enzyme action at low temperature see the review by HEPBURN (**6**); and the following papers: PHILLIS and MASON (**10**), JOSLYN and SHERRILL (**7**), and KERTESZ (**8b**). Extreme care should be exercised when samples frozen without heat treatment are thawed: they should be defrosted in alcohol, when extractions are to be performed, or heated to inactive enzymes immediately after defrosting to minimize effects upon the composition. Heat treatment and drying, and preservation in alcohol after heating are preferable to preservation by freezing.

C. Heat drying.—This method was outlined in our original recommendations. Small samples may be dried quickly at 60° C. *in vacuo,* but it must be recognized that enzymes are not thus inactivated. The Committee recommends, with particular reference to larger samples, prompt inactivation of enzymes by heating to 80° C. for 10 min., followed by drying at 70° in a well ventilated oven. This implies, of course, assurance that the innermost cells of the tissue acquire the specified temperature. Dried tissues should be stored in sealed containers to prevent absorption of water, but this applies more particularly when enzymes have not been inactivated.

EXTRACTION.—The use of alcohol to give at least 80 per cent. strength, and with heating, is to be preferred as an extractive method where one is concerned primarily with the determination of sugars. Without heating, as in the use of a Soxhlet extractor, solubility is greatly diminished. The apprehension expressed earlier regarding possible hydrolysis of sucrose by tissue acids was apparently unjustified. DENNY (**4b**) found the use of $CaCO_3$ to prevent this action generally unnecessary.

Owing to their lesser solubility, it may be expected that amino acids and other simpler nitrogenous constituents will require more extended extraction than the sugar fraction. For this type of extraction excessive heating of the extract should be avoided. It is well to use successive smaller portions of solvent, limiting the temperature to 80° and its effective time to 10 min. This treatment should largely inactivate enzymes, while hastening the precipitation of colloids.

Increasing yields of extractives as reported by users of the autoclave should be viewed reservedly until the extent of alteration by hydrolysis or otherwise is known. The use of cytolyzing agents in preparation of fresh tissue when it is desired to obtain undiluted sap tends, of course, to release enzymes and calls for speed and low temperatures in the operation. This same risk attends grinding of the tissue with water at ordinary temperatures, but the high solvent power of water must be considered an important asset. STUART (private communication from the University of Maryland) as an average for 9 species extracted 40 per cent. more non-protein nitrogen

with cold water than with hot alcohol. On the other hand, LOOMIS (private communication from Iowa State College) reports a distinct end-point in extraction by alcohol for a considerable variety of tissue samples. It may prove feasible to increase the solvent efficiency of alcohol by dilution to 50 per cent., while retaining to a large degree its stabilizing capacity. To avoid appreciable changes of composition in the treatment of large samples one may adopt extraction by hot water, in the manner practiced by VICKERY and PUCHER (**12**) on tobacco leaves. This procedure combines prompt inactivation of enzyme with high solvent efficiency.

DEFOAMERS AND DEEMULSIFIERS.—Some extracts, more particularly those obtained by pressure only or the use of cold water, tend to froth freely. This property disturbs the establishing of volumes, aeration procedure, and the Van Slyke determination of amino nitrogen. A few drops of ether cut the foam for making to volume, but this agent is lost, of course, in aeration. SCHLENKER (**11**) recommends for aspiration procedure the addition of paraffin dissolved in xylene. Heavy alcohols serve the same purpose but their efficiency is gradually lost. Primary caprylic alcohol is used in the Van Slyke determination, although the secondary form, also designated as methyl-n-hexyl carbinol, seems to be preferred. Attention may be called to directions (**5**) by which secondary caprylic alcohol can be prepared readily from castor oil. H. A. CONNER (private communication, Department of Plant Pathology, University of Wisconsin) recently discovered that a solution of castor oil in ethyl alcohol provides a very efficient foam breaker. Although but a few drops are employed, these agents should be examined as possible sources of a high blank in the microdetermination of alpha-amino nitrogen.

It has been customary to depend upon the water attracting power of certain salts for the purpose of breaking troublesome emulsions. When the presence of nitrates prohibited the introduction of chlorides PHILLIPS (private communication from University of New Hampshire) found it possible to substitute 0.3 per cent. $CaCl_2$ by the molecular equivalent of $MgSO_4$. CHANNON and FOSTER (**1**) overcame persistent emulsions in washing ethereal solutions of lipides by introducing acetone with NaCl (100 parts water, 40 parts acetone, 5 parts salt).

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DETERMINATION OF CARBOHYDRATES[1]

This report is supplementary to the previous reports of the committee on soluble carbohydrates and polysaccharides (Plant Physiol. **2**: 91–97; 195–204. 1927), and should be used in conjunction with the original reports.

CLEARING METHODS.—The first report recommended clearing sugar solutions with saturated neutral lead acetate solution and deleading with potassium oxalate. MCGILLIVRAY (**10**) finds that clearing does not affect the reducing power of tomato extracts and consequently can be omitted with this material. Undoubtedly clearing of many other materials is more a matter of convenience in handling than of necessity. Whenever sugar solutions do not give a lowered reducing value upon clearing with neutral lead acetate, and can be conveniently handled without clearing, this operation may be considered to be dispensable. Clearing is necessary (a) whenever tannins or similar reducing impurities are present in the extract, and (b) whenever dextrin or inulin extracts (containing, as they ordinarily do, gums and soluble hemicelluloses) are to be hydrolyzed with acid. Note that the clearing is given before the acid hydrolysis.

LINCOLN (University of Maryland)[2] reports that basic lead acetate has been more satisfactory than neutral lead for clearing wood extracts. Basic lead, properly handled, can be used for solutions which do not contain fructose or inulin, but the safety margin is narrow. LOOMIS (**9**) has shown that the more complete clearing with basic lead does not affect the purity of the copper precipitate, although LINCOLN reports better crystallization. The use of basic lead, neutral lead, or no clearing can be justified for different materials. The committee recommends the use of neutral lead clearing as a safe procedure, other methods to be used only after testing.

DELEADING.—The last revision of the A.O.A.C. methods (**1**) requires the anhydrous sodium or potassium oxalate for deleading solutions made to volume before filtering out the lead precipitate. The water contained in crystallized potassium oxalate introduces a dilution error under these conditions. The objection of ENGLIS and TSANG (**5**) to oxalate deleading is based upon determinations which gave a very heavy lead precipitate, and apparently they did not allow time for the oxalate precipitate to crystallize. LOOMIS (**9**) worked with plant extracts instead of the pure sugar solutions used by ENGLIS and TSANG, and found that reducing sugars were lost in the clearing rather than in the lead oxalate precipitate as reported by the latter workers. Sugars held in the lead oxalate precipitate of ENGLIS and

[1] This report was assembled by W. E. LOOMIS for the committee on chemical methods: Z. I. KERTESZ, W. E. LOOMIS, T. G. PHILLIPS, W. E. TOTTINGHAM, chairman.

[2] Location in parentheses indicates private communication.

TSANG were largely removed by cold water washing, but much of the sugar held in the basic lead precipitate of a leaf extract was recovered only by treating the mass with hydrogen sulphide. These results offer no assurance that reducing sugar losses due to the use of basic lead clearing can be overcome by the proper choice of deleading reagent.

SUGAR DETERMINATIONS.—An increasing number of plant chemists are using the SHAFFER and HARTMANN (**14**) sugar method. CLEMENTS (**4**) reports that it has proved more reliable on pea plant extracts than the MUNSON and WALKER (**12**) method. MARSH and DICKSON (**11**) have compared the two methods for sugar determinations on wheat and soy beans. They found the SHAFFER and HARTMANN method to give uniformly higher results, the difference varying with the plant from 5 or 10 to as much as 43 per cent. and becoming less as the plants aged. The percentage deviation was also less for total than for reducing sugars. They state, "It seems possible that some substance which affects the accuracy of the SHAFFER-HARTMANN method is removed or changed by the added acids or by their salts. Also such a material appears to vary with the age of the plant. . . . Further work along this line might reveal that the addition of certain salts would increase the accuracy of the SHAFFER-HARTMANN procedure for use in plant analysis." Poor end points were also encountered in some solutions with the SHAFFER-HARTMANN method.

The convenience of the SHAFFER-HARTMANN method recommends its use whenever possible and it is to be hoped that means may be found to increase the general reliability of the method. It would seem at present that the determination of reduced copper after filtering and washing is still the safest method for general use on plant tissues.

The BERTRAND method (**2**) of determining reducing power is commonly used in Europe, and the recent publication (**8**) of complete tables for use with the method adds to its usefulness. Many American workers prefer to follow the MUNSON-WALKER reducing conditions for which copper-sugar tables are generally available, and to use the BERTRAND method of determining copper by dissolving the cuprous oxide in acid ferric sulphate or ferric alum and titrating the reduced iron with potassium permanganate. Directions for the copper determinations may be found in KERTESZ's article (**8**) or in the methods section of MATHEWS' Physiological Chemistry.

HYDROLYSIS OF SUCROSE.—WEBSTER (**15**) has reported difficulties with the invertase method and suggests the use of citric acid inversion for sucrose. At the Iowa State College laboratories an invertase method supplied by KRAYBILL has been used in class and research work by some 20 graduate students with complete satisfaction. Four 50-ml. portions of the cleared and deleaded sugar extract are pipetted into 400-ml. beakers. Two of the samples are used for the duplicate determination of reducing sugars.

One or two drops of methyl red solution are added to the other samples, then two to four drops of 10 per cent. acetic acid to bring to the acid color of methyl red, and two to four drops of a 1 per cent. solution of Wallerstein invertase scales. The solutions are allowed to stand for 2 to 24 hours, Fehling solution is added and reducing sugars determined. The blanks for the sucrose determinations should receive the same dose of invertase solution as is used for the samples. Two lots of invertase scale have been used and both have given complete inversion of 50 mg. of sucrose in 50 ml. of solution in two hours at 25° C. The 1 per cent. invertase solution has been kept under toluene and on ice for two months. It is probable that some lots of invertase might require larger quantities of the enzyme or more than a 2-hour minimum period. This point should be checked by varying the inversion time with replicate samples.

With such small quantities of enzyme in place of the heavy dosages sometimes recommended, the expense of the method is negligible and difficulties due to the addition of large quantities of colloidal material are avoided. The use of the entire inverted sample without neutralization or transfer is convenient and time saving, and the specificity of the method recommends it for general use.

SEPARATION OF GLUCOSE AND FRUCTOSE.—Most published analyses of plants report sugars as reducing or non-reducing. When invertase is used for inversion it is considered permissible to multiply the increased reduction, calculated as invert sugar, by 0.95 and report as sucrose. The separate estimation of glucose and fructose in the free reducing sugars is also frequently desirable.

PHILLIS and MASON (**13**) have used a modified iodine method for glucose. Glucose was oxidized according to the procedure of HINTON and MACARA (**6**); the solution was then made acid and excess iodine removed with sodium sulphite. Loss of reducing power with the treatment was recorded as glucose, and the reducing power after treatment as fructose.

In the Iowa State College laboratories JACKSON's (**7**) method for fructose has been found convenient. Fructose is determined by reduction of OSTS' copper carbonate solution for 75 minutes at 55° C., and glucose by difference of fructose and reducing sugars after correcting for the reducing action of the glucose in the fructose determination. Fructose added to plant extracts has been recovered by this method and hydrolyzed sucrose has given the expected 50 per cent. fructose.

DEXTRINS.—LOOMIS (Iowa State College) reports that two dextrin fractions and no starch have been isolated from the leaves of corn. The first fraction is extracted by 10 per cent. cold alcohol and the second by boiling water. The end points of the two extractions are good although continued extraction by either method apparently results in some hydrolysis of

underlying materials. Extractions are conveniently made by suspending the sample of finely ground (200-mesh) residue, previously extracted with 80 per cent. alcohol, in the 10 per cent. alcohol or boiling water, stirring occasionally for 30 minutes, centrifuging, and decanting the extract. Four extractions have been adequate for corn leaf and stalk tissues. The extracts are cleared with neutral lead acetate, brought to volume, filtered, deleaded, and aliquots hydrolyzed with 1 per cent. hydrochloric acid for one hour at 15 pounds' pressure. Hydrolysis in the autoclave under these conditions has been equivalent to refluxing for two and one-half hours with 2 per cent. hydrochloric acid and is much more convenient. Reducing sugars are calculated as glucose and multiplied by 0.90 to obtain dextrins.

STARCH.—LINCOLN (University of Maryland) finds that fine grinding is necessary for effective removal of starch by enzyme action, and the same report is made by LOOMIS (Iowa State College). At the Iowa laboratory all starch and dextrin samples are ground in a ball mill and passed through a 200-mesh sieve.

KERTESZ (New York Agricultural Experiment Station) reemphasizes a point made in the first report of this committee, that total acid hydrolyzable material should not be reported as starch. Enzyme digestion and clearing before acid hydrolysis is recommended. Clearing of the enzyme extract removes soluble hemicelluloses and gums which would otherwise be hydrolyzed and found as starch. Taka-diastase, animal diastase, and saliva are used for starch hydrolysis. The diastatic power of fresh saliva varies greatly and should be checked. It appears to be preferable to collect saliva before, rather than just after meal time. Both taka-diastase and animal diastase tend to give high results because of the presence of enzymes other than amylase.

CARR (3) has published the results of some very interesting experiments indicating the possibility of dividing starch into fractions which are apparently of economic and possibly of physiological importance. CARR finds that 45 per cent. formic acid separates soluble starch, colloidal starch, and cellulose in ground wheat. Since gums are reported as undissolved, the method would appear to have possibilities both in fractioning total starch and in the routine separation of starch and hemicelluloses.

HEMICELLULOSES.—The physiological significance of these materials has not yet been established and for this reason they should not be determined with the starch or dextrin. The acid hydrolysis of hemicelluloses ordinarily does not give an end point, as measured by the production of reducing substances, but instead drops to a constant low value which probably represents the digestion of cellulose. The hydrolysis conditions should be such that this relatively stable portion of the curve is reached at the end point. LOOMIS (Iowa State College) reports that autoclaving the starch and

dextrin free samples with 1 per cent. hydrochloric acid for one hour at 15 pounds gives a good end point for corn leaf material. As already stated, this treatment has been found to be equivalent to two and one-half hours of refluxing with 2 per cent. acid. In using the method, the powder from which starch and dextrins have been removed is transferred to 250-ml. flasks with 100 ml. of water; 2.7 ml. of concentrated (38 per cent.) hydrochloric acid are added, and the samples autoclaved with a small funnel for a reflux. The extract is then filtered into a 250-ml. volumetric flask and the residue washed. The acid is nearly neutralized with NaOH (m.r.), the solution is made to volume, and its reducing power determined. Clearing is not required for this fraction since the common interfering substances are hydrolyzed by the treatment.

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DETERMINATION OF NITROGEN IN RELATIVELY SIMPLE COMPOUNDS[1]

Total nitrogen

A. In the absence of nitrates.—Any of the recognized modifications of the Kjeldahl method may be used. The amounts of catalyst and acid required vary with the amount of organic matter to be oxidized. For 50-cc. aliquots of the alcoholic extract of fruit spurs, containing 1–3 mg. N, 15 cc. concentrated H_2SO_4 and 5–7 gm. of a mixture of 10 gm. K_2SO_4 and 1 gm. $CuSO_4 \cdot 5H_2O$ were adequate. Frothing is likely to be severe as the last of the water or alcohol is driven off. By careful regulation of the flames and occasional shaking it has been possible to make 30 digestions at once without loss. For the final titration the mixed methyl red-methylene blue indicator proposed by Johnson and Green (8) is excellent.

B. In the presence of nitrates.—The use of the salicylic acid method devised by Ranker and described in the previous report (27) is troublesome because of the necessity of evaporating an exactly neutral portion of the extract to dryness in the Kjeldahl flask before adding the reagents. This difficulty is overcome by the reduced iron method, which was adapted to plant materials by Pucher, Leavenworth, and Vickery (11). The reduction may be carried out in either aqueous or alcoholic solution. The conditions for the reduction prescribed by Pucher *et al.* should be followed exactly. Those for the subsequent digestion may be modified somewhat. For 25-cc. aliquots of the alcoholic extract of tomato leaves, containing 1–5 mg. of nitrogen, 25 cc. of concentrated H_2SO_4 and 5–7 gm. of the catalyst mentioned above have been found adequate.

Doneen (4) has described a micromethod using salicylic acid. The digest is Nesslerized directly in the presence of gum arabic, and the nitrogen is determined colorimetrically. Preliminary evaporation of a portion of the extract is necessary. As approximately 1 mg. of nitrogen is required for a satisfactory determination, there would seem to be little advantage in this regard over the reduced iron method.

Fractionation of the nitrogen

The particular methods to be adopted depend on the nature of the material, the method of extraction, and the purpose of the study. Some methods that have proved useful are cited below. Choice must be made in each case of those best adapted to the materials concerned.

[1] This report was assembled by T. G. Phillips for the committee on chemical methods: Z. I. Kertesz, W. E. Loomis, T. G. Phillips, W. E. Tottingham, chairman.

The presence of alkaloids or cyanogenetic glucosides complicates matters considerably. VICKERY and his associates (**12, 22, 23, 25**) have developed methods applicable to tobacco, and DAVIDSON and SHIVE (**3**) have studied the distribution of nitrogen in the presence of a cyanogenetic glucoside.

STUART (**16**) has shown that 80 per cent. alcohol does not extract as much soluble non-protein nitrogen from plant parts as water does, and both he and WEBSTER (**26**) have found changes in the distribution of nitrogen on standing in alcoholic solution. It seems possible that 50 per cent. alcohol might prove satisfactory for extraction, and that changes might be avoided by removing the alcohol from the aliquots used for nitrogen distribution as soon as possible after extraction is complete. Further studies of these points are needed.

Semi-micromethods for ammonia, amide, and nitrate nitrogen, applicable in case extracts can be concentrated sufficiently so that 10-cc. portions contain determinable amounts of these forms of nitrogen, were mentioned in the previous report of this committee (**27**). They are described in detail by STUART (**15**). When portions of alcoholic extracts are freed from alcohol and taken up in water, some of the water insoluble material may form an emulsion. In many cases shaking with chloroform and allowing to stand overnight yields a clear water solution. With some materials a flocculating agent is required. Magnesium sulphate as used by STUART is satisfactory. It is not as effective as calcium chloride, but the latter cannot be used in the presence of nitrates.

AMINO NITROGEN

This is usually determined in the VAN SLYKE microamino apparatus (**18, 19**). The pipette holds a 2-cc. sample but 4 cc. may be used. An improved reaction chamber has been devised by KOCH (**9**). The sample must be free from alcohol, and the portion used should contain at least 0.2 mg. of amino nitrogen. It may be desirable occasionally to use the larger reaction chamber with the micro burette, so that larger samples, up to 10 cc., can be taken. Description of the apparatus and technique and the necessary tables will be found in the papers cited and in MATHEWS' Physiological Chemistry. VAN SLYKE's manometric equipment may be used for measuring the nitrogen liberated (**20**). The equipment and method are described also by PETERS and VAN SLYKE (**10**).

If ammonia is present in the sample in appreciable amounts it must be removed before the determination of amino nitrogen is made. STUART (**16**) has found that other substances, among which the polyhydric phenols are important, interfere with the method by yielding excessive quantities of gas which is measured as nitrogen. These as well as ammonia are removed or

denatured best by vacuum distillation of the sample at 45° with a slight excess of calcium oxide.

Ammonia nitrogen

There are two points in connection with this determination which should be emphasized.

1. The treatment of the sample for the liberation of ammonia must be mild in order to avoid the formation of ammonia from compounds other than ammonium salts.

2. Some plants contain appreciable quantities of other volatile bases which are carried over with ammonia in the usual methods of aeration or distillation.

For the removal of ammonia from 10 cc. or less of solution in the determination of urea, Van Slyke and Cullen suggested aeration with an equal volume of 52 per cent. K_2CO_3. An adaptation of this method to plant extracts was described in the previous report of this committee, and by Stuart (**15**).

For larger volumes Sessions and Shive (**14**) used Na_2CO_3 and NaCl with aeration.

Vickery and Pucher (**22**) removed NH_3 from tobacco extracts by a short distillation at atmospheric pressure with magnesium oxide. Schlenker (**13**) has found this method to give somewhat excessive results with other materials. If distillation is to be used, it appears safer to carry it out under reduced pressure at 40°–50°, either with magnesium oxide, or as suggested by Van Slyke (**17**) with calcium hydroxide and alcohol. If the ammonia is determined by titration, volatile bases other than ammonia will be included. Schlenker (**13**) has found this error negligible with a number of plants. With others the error may be comparatively large. Vickery and Pucher (**22**) avoid this difficulty by an adaptation of the permutit method of Folin and Bell. The ammonium ions are taken up by permutit from a slightly acid solution. After washing, the ammonia is liberated by sodium hydroxide and determined colorimetrically with Nessler's reagent.

Schlenker has removed ammonia from plant juices by direct treatment with permutit. As some pigments are absorbed also, aeration from the permutit is necessary before the ammonia can be determined colorimetrically. Various combinations of absorption with permutit and either colorimetric or titrimetric determination are possible. If volatile bases other than ammonia are present in appreciable amounts, the permutit colorimetric combination is to be recommended, since Vickery and Pucher (**22**) have found that these bases either are only slightly absorbed by permutit, or in small amounts do not affect Nessler's reagent.

AMIDE NITROGEN

This is measured as the increase in ammonia nitrogen caused by mild acid hydrolysis. The exact conditions for the hydrolysis have not been standardized.

Hydrochloric acid has been used most frequently, but VICKERY and PUCHER (**24**) and CHIBNALL and MILLER (**2**) have shown that large errors are caused by this acid if nitrates are present in appreciable amounts. The other phase of this difficulty, that is, the loss of nitrates on boiling with HCl, was noted in the previous report of this committee, and the use of H_2SO_4 was suggested if the amide hydrolysis was to be followed by the determination of nitrates.

Until some standardization of the method is achieved, the conditions described in the previous report may be repeated. These were boiling under a reflux condenser for 2.5 hours with 6 per cent. HCl. As nitrates are usually present, it is best to use 10 per cent. H_2SO_4 instead of the 6 per cent. HCl, as this furnishes about the same actual acidity. The addition of 0.6 cc. concentrated H_2SO_4 to 10 cc. of the solution gives this result. From the total ammonia nitrogen as determined by one of the methods just referred to, the original ammonia nitrogen is subtracted and the difference is considered amide nitrogen.

This method has given satisfactory recovery of known amounts of the amide nitrogen of asparagine in the presence of nitrate, ammonia, amino and peptone nitrogen.

PUCHER[2] finds that the amide nitrogen of glutamine is hydrolyzed much more easily than that of asparagine, and that glutamine occurs in plants more generally than had been supposed. It is possible that some of the nitrogen usually determined as ammonia may come from this source.

NITRATE NITROGEN

Three types of methods have been used for carrying out this determination:

(1) Reduction in alkaline solution with Devarda's alloy and determination as ammonia.

(2) Reduction in acid solution with iron and determination as ammonia.

(3) Colorimetric methods.

In addition to the discussion of the use of Devarda's alloy in the previous report, it should be noted that SESSIONS and SHIVE (**14**) determine nitrate nitrogen in a sample in which ammonia nitrogen has been determined, by aerating, after making the solution eighth normal with NaOH and adding 1 gm. of Devarda's alloy. The semi-micromethod outlined in

[2] Reported at the meeting of the New England Section of the American Society of Plant Physiologists, Amherst, Massachusetts, May 25–26, 1934.

the previous report has given satisfactory recovery of known amounts of nitrate nitrogen in the presence of ammonia, amide, amino, and peptone nitrogen. Loomis[3] reports the finding of appreciable amounts of nitrate nitrogen by this method in sweet clover roots which were shown by qualitative tests to contain no nitrates.

It would seem probable that the solution left after the determination of amide nitrogen by any of the usual methods could be used for the nitrate determination. It should be transferred to a Kjeldahl flask, enough alkali added to make a final volume of 300 cc. tenth normal with NaOH, and boiled down to a small volume in order to liberate any ammonia that could be set free by the alkali. After dilution to 300 cc. and the addition of 1 gm. of Devarda's alloy, distillation could be carried out in the usual way on a Kjeldahl rack with an efficient scrubber bulb.

Vickery and Pucher (23) have described the determination of nitrate nitrogen in tobacco following steam distillation from alkaline solution to remove nicotine and other volatile bases. The nitrate nitrogen is reduced to ammonia in acid solution by reduced iron. A blank or comparison run must be made without the iron. The difference in the amount of ammonia nitrogen obtained in the two determinations represents the nitrate nitrogen of the sample. In samples containing only small amounts of volatile base, the method may be used without the preliminary steam distillation.

Pucher, Vickery, and Wakeman (12) recommend the extraction by ether of nitric acid from plant material that has been acidified to pH 0.7 to 0.9 by H_2SO_4. Extracts may be treated in this way by concentration and absorption with asbestos before extraction. Nitrate nitrogen is determined in an aliquot of the extract by the reduced iron method, after making alkaline and removing the ether. A comparison run without the reduced iron must be made in this case also.

Several studies have been made recently of the phenol disulphonic acid method for the determination of nitrates in plant juices or extracts. Emmert (5) has developed a somewhat simplified method for use with tomato and lettuce, and Holtz and Larson (7) have described one for wheat. Frear (6) has revised the method of Gilbert in the light of the criticism of various workers, and has proposed a method which would appear to be applicable generally to plant juices or aqueous extracts. If alcoholic extracts are used the preliminary clearing with lead acetate recommended by Burrell and Phillips (1) is likely to be necessary. According to Emmert the use of NaOH instead of NH_4OH for making the phenol disulphonic acid mixture alkaline avoids off colors with small amounts of sugar, and so would make unnecessary the peroxide treatment used by Burrell and Phillips. The evidence available appears to preclude the use of charcoal as a clearing agent.

[3] Correspondence.

The following items may be added to the brief description in the previous report of methods for the determination of nitrogen in other forms.

VICKERY (**21**) has discussed the basic nitrogen of plant extracts and has shown that no methods that have appeared as yet for the determination of this fraction yield results that can be interpreted definitely in chemical terms.

VICKERY *et al.* (**25**) determined peptide nitrogen in extracts of tobacco leaves by measuring the increase of amino nitrogen resulting from boiling 20 hours with concentrated hydrochloric acid. They note that this method is not reliable in the presence of nitrates. For this reason the determination was made on the residue from which nitric acid had been extracted by ether as described above. Ammonia formed during the hydrolysis was removed by distillation with magnesium oxide before amino nitrogen was determined.

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BRIEF PAPERS

FORMATION OF CHLOROPHYLL AND THE BEGINNING OF PHOTOSYNTHESIS[1]

In 1910 IRVING (4), using *Vicia faba* and *Hordeum*, came to the conclusion that etiolated leaves do not possess an appreciable power for carrying on the photosynthesis of carbon dioxide until they have been exposed to light sufficiently long so that they are almost fully green. WILLSTÄTTER and STOLL (5) came to a contrary conclusion, since they found leaves with but a small portion of their full chlorophyll content capable of carrying on carbon assimilation quite rapidly. BRIGGS (1) believed these experimental discrepancies were due chiefly to the inequality in the age of the leaves used and to the variation in the lapse of time from the greening to the measurement of the photosynthetic activity. DASTUR (2) thought the discrepancies noted might be due to loss of water or irregularities in the age of the leaves, together with the difficulty of selecting uniform samples.

Experimental methods

Seeds of *Zea mays* were planted on moist soil in a dark room. The temperature ranged about 26° ± 2° C. At the end of twelve days the unfolded leaves were collected and floated on tap water kept at 24° ± 1° C.

TABLE I

EXPOSURE OF ETIOLATED LEAVES OF *ZEA MAYS*

TIME OF EXPOSURE	TEMPERATURE	AGE FROM PLANTING	COLOR OF LEAVES TO EYE	EVOLUTION OF OXYGEN
min.				
0	24° ± 1°C.	12 days	Yellow	Negative
15	"	"	"	"
30	"	"	"	"
45	"	"	"	"
60	"	"	"	"
75	"	"	"	"
90	"	"	"	"
105	"	"	Yellow-orange	"
120	"	"	"	"
135	"	"	Slightly green	Trace
150	"	"	"	Positive

[1] Contribution from the C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis.

A 100-watt mazda lamp was placed above the water bath at a distance of 18 inches. Samples of the leaves were tested for the evolution of oxygen before exposure to light and every 15 minutes for a period of three hours. Luminescent bacteria were used to detect the presence of oxygen (3). Table I reports a single experiment.

Other experiments with *Zea, Triticum,* and *Avena* showed variations of from 15 to 30 minutes in the time when the first evolution of oxygen could be detected. However, the evolution of oxygen always began at about the same time that the eye could detect the appearance of a green color in the leaf. Acetone extracts of the same crop of *Zea* leaves before exposure to light gave the following absorption spectrum:

Bands in ether	Shadow	I	Shadow	II	Shadow	E.A.
	662.0	624.5	601.5	572.4	532.5	511.0

Intensity, I, II

The absorption spectrum after acidification of the above solution with dilute HCl was as follows:

Shadow	Shadow	I	II	III	Shadow	E.A.
666.0	638.0	596.1	582.9	565.3	528.8	513.5

Intensity, III, II, I

When etiolated leaves were exposed to the light of a 100-watt bulb at a distance of 8 inches for two minutes, a representative absorption spectrum was as follows:

Bands in ether	I	II	III	Shadow	IV	E.A.
	666.3	624.5	605.2	565.0	536.3	517.1

Intensity, I, IV, III, II

Formation of the 666.0 band of chlorophyll could be detected easily after 10 seconds' exposure to the light. After exposure of etiolated leaves for 150 minutes a typical chlorophyll absorption spectrum was obtained.

Discussion and conclusions

It is not easy to draw definite conclusions concerning the beginning of photosynthesis when respiration must be taken into account. It seems fair to conclude, however, that the first traces of the evolution of oxygen indicate the time when an excess is produced within the plant, and that at least the process of photosynthesis is going on when oxygen is evolved.

From the experimental evidence obtained it is clear that the evolution of oxygen may begin very soon after chlorophyll is formed in sufficient amount to render the young etiolated leaf green to the eye, and is not delayed

until the leaf is almost fully green. On the other hand, an examination of the absorption spectrum of extracts of etiolated leaves after exposure of from ten seconds to two minutes shows that some chlorophyll is formed long before the evolution of oxygen begins.

No attempt was made to test for the absorption of carbon dioxide during the experiments.—O. L. Inman, *Antioch College, Yellow Springs, Ohio.*

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SALT CONCENTRATION AND REVERSIBILITY OF ICE-FORMATION AS RELATED TO THE HARDINESS OF ALFALFA

In a previous paper (**1**) it was shown that in four varieties of winter wheat, the concentration of soluble electrolytes in the crowns decreased as the winter season approached. The ice formed in the tissues was determined by the calorimetric method and the unfrozen water computed. The concentration of soluble salts in this unfrozen portion of water was shown to decrease greatly as the plants became more hardy.

In the experiment to be described, plants of three varieties of alfalfa were grown to an age of about six months (August 24 to February 7) in the greenhouse in 6-inch pots. The soil was fertilized and inoculated. Approximately ten plants were grown in each pot. The varieties Grimm, Utah Common, and Hairy Peruvian were selected, since these make the characteristic short day growth. The tops of the Grimm plants remained short, those of the Hairy Peruvian were about 1 foot long, and those of the Utah Common were intermediate.

Samples of roots were severed from the tops at the crown. The roots were washed and the hair like roots stripped off as completely as possible. The samples were rinsed quickly in distilled water, cut into pieces about

2 cm. long, and allowed to dry under moist toweling with occasional mixing. Four samples of 20 gm. each were prepared from each variety on each date, in addition to a sample prepared for determination of dry matter. Because of the considerable amount of fine rootlets formed when the plants are grown in the greenhouse, especially on the Grimm variety, it was never felt that the determination of percentage dry matter was perfectly accurate. It seemed impossible to determine precisely when the samples had become superficially dry, since the fine rootlets formed moist compact masses when the main roots, perhaps 3 mm. in diameter, were dry. This experimental difficulty may be responsible for the seemingly aberrant figures appearing in the data.

TABLE I

VALUES OF SPECIFIC CONDUCTIVITY, IN RECIPROCAL OHMS $\times 10^5$- (25°C.), OF THE EXTRACTS FROM 20 GM. OF ALFALFA ROOTS INTO 300 CC. OF WATER. SPECIFIC CONDUCTIVITY FOLLOWING FREEZING ALONE IS INDICATED AS "FREEZING INJURY" WHILE THAT FOLLOWING SUBSEQUENT HEATING IS SHOWN AS "TOTAL SALTS"

Sample	Frozen −6°C.				Frozen −9°C.				
	Freezing injury		Total salts		Freezing injury		Total salts		
	Frozen		Frozen		Frozen		Frozen		
	Once	Twice	Once	Twice	Once	Twice	Once	Twice	
	No days' hardening								
Grimm	44.3	50.8	.	75.0	58.2	61.0	74.2	73.8	
Utah Common	50.5	50.0	71.0	71.0	59.2	61.0	71.0	71.0	
Hairy Peruvian	53.4	55.2	72.5	69.4	59.2	63.4	70.2	69.4	
	10 days' hardening								
Grimm	34.9	32.7	74.2	74.7	48.3	55.7	74.2	75.0	
Utah Common	42.2	42.2	71.7	70.2	54.7	57.2	70.2	69.4	
Hairy Peruvian	43.3	55.2	71.0	71.0	59.2	61.0	70.2	71.7	
	20 days' hardening								
									Average total salts
Grimm	34.5	35.7	71.7	75.0	56.5	58.2	72.5	74.2	74.1
Utah Common	42.2	47.2	68.7	71.0	58.7	61.5	70.6	71.7	70.6
Hairy Peruvian	52.0	56.1	70.6	70.6	62.0	67.2	69.8	73.3	70.8

The procedure followed is described in detail in a recent paper (**1**). The samples were wrapped in tinfoil, placed in tapered, tubular copper containers, and stored at 2° C. overnight to insure a definite temperature at the beginning of freezing. They were frozen by immersion in an alcohol-ice bath for two hours, at $-6°$ C. or $-9°$ C. The duplicate in each case was thawed for two hours at 2° C. and refrozen in the original way. The quantity of ice was determined in each sample immediately on withdrawal from the freezing bath, using the calorimetric method. Injury was estimated from the exosmosis of electrolytes into the water (300 cc.) used in the calorimeter, after a period of 22 hours at 2° C. (**2**, **3**). The samples were then heated to boiling and exosmosis continued for 24 hours, when the total extracted electrolytes were determined electrometrically.

Three series of determinations were made. The first was made on roots of plants direct from the greenhouse. The rest of the pots were placed at

TABLE II

DATA OF ICE-FORMATION COMPUTED FROM CALORIMETRIC MEASUREMENTS. THE "SALTS PER GM. UNFROZEN WATER" IS COMPUTED BY DIVIDING THE "AVERAGE TOTAL SALTS" BY THE VALUE FOR "GRAMS UNFROZEN WATER" FOR EACH SAMPLE IN TURN

Sample	Frozen −6°C.				Frozen −9°C.				Percentage dry matter
	Grams unfrozen water		Salts per gm. unfrozen water		Grams unfrozen water		Salts per gm. unfrozen water		
	Frozen		Frozen		Frozen		Frozen		
	Once	Twice	Once	Twice	Once	Twice	Once	Twice	
	No days' hardening								
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%
Grimm	5.39	4.15	13.75	17.85	3.28	3.29	22.5	22.6	42.2
Utah Common	5.39	5.39	13.1	13.1	3.73	3.73	18.9	18.9	42.2
Hairy Peruvian ...	6.32	4.91	11.2	14.5	4.25	3.38	16.7	20.9	35.5
	10 days' hardening								
Grimm . .	6.82	5.77	10.9	12.85	5.04	2.91	14.7	25.5	39.2
Utah Common ..	6.01	5.58	10.8	12.65	3.73	3.88	18.9	18.2	41.6
Hairy Peruvian .	7.27	5.04	9.73	14.1	4.04	3.54	17.5	20.0	35.9
	20 days' hardening								
Grimm	7.01	6.36	10.6	11.65	4.12	3.72	18.0	19.9	38.7
Utah Common	6.72	5.98	10.5	11.8	4.20	3.45	16.8	21.2	40.3
Hairy Peruvian ...	4.16	3.94	17.1	18.0	2.55	0.75	27.8	94.5	40.7

a temperature of 2° C. with continuous illumination with mazda lamps. After 10- and after 20-day periods at this temperature, further samples were taken. Tables I and II summarize the data for the experiment.

It will be observed that in the varieties which hardened most, less water was frozen and more left unfrozen when the plants were in the hardy condition than when they were tender. The percentage dry matter in the samples of the hardier varieties tended to decrease slightly during the period in the cold room. These were already "short day" plants, low in vegetative vigor and high in dry matter. This behavior is in sharp distinction from that of winter wheat under the same conditions, where percentage dry matter increases markedly. In the case of the samples of alfalfa, there appears to be no loss of soluble electrolytes during the period in the cold room. This, too, is sharply different from the behavior of winter wheat. Since, however, there is more water left unfrozen when the plants are in the hardy condition than when they are tender, the concentration of electrolytes in the unfrozen sap is less in the hardened plants. This was true to a more marked degree in the case of winter wheat, which also hardens more than alfalfa and will survive lower temperatures.

The data are by no means so clear-cut as those with the winter wheat. The evidence seems conclusive, however, that there is no loss of electrolytes in the case of any of the varieties of alfalfa during hardening itself. It would be worth while to repeat this experiment with alfalfa plants from the field throughout the autumn and early winter period, as was done with winter wheat. Under such conditions the roots are relatively free from the troublesome hairlike roots.

These data, inadequate though they are, are presented at this time since no opportunity to repeat the experiment seems available.—S. T. DEXTER,[1] *University Farm, St. Paul, Minnesota.*

LITERATURE CITED

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2. ———, TOTTINGHAM, W. E., and GRABER, L. F. Preliminary results in measuring the hardiness of plants. Plant Physiol. **5**: 215–223. 1930.

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[1] National Research Council Fellow.

NOTES

Summer Meeting.—The summer meeting of the American Society of Plant Physiologists will be held in Minneapolis and St. Paul from June 24–29. Headquarters for the meeting are at the Plant Pathology and Botany Building, University Farm. The program is being arranged by Dr. R. H. Landon, Chairman of the Minnesota Section, and Ralph W. Lorenz, Secretary, Division of Forestry, University Farm, St. Paul. The tentative program has been arranged as follows:

Monday A. M.	Registration at the A. A. A. S. headquarters.
Tuesday A. M.	Joint session, Section O of the A. A. A. S., and symposium on "Improving the germplasm of domestic plants and animals," with addresses by Dean W. C. Coffey, Hon. H. A. Wallace, J. L. Lush, and Olaf Aamodt.
Tuesday P. M.	Discussions and inspection of laboratories and demonstration of research work in progress in the physiological laboratory, Department of Botany (Minneapolis Campus) and Section of Plant Physiology (University Farm); also laboratories of the Shelter Belt Project of the Lake States Forest Experiment Station at University Farm.
Tuesday evening	A picnic supper on the St. Croix river has been arranged by Dr. and Mrs. R. B. Harvey.
Wednesday A. M.	Session for presentation of papers of the A. S. P. P., Room 102 Horticulture Building, University Farm. Symposium for presentation of papers on dormancy, after-ripening, and germination of seeds.
Wednesday P. M.	Continuation of morning symposium if necessary, with the remainder of the afternoon for papers of general interest.
Thursday A. M.	Field trip with plant pathologists and horticulturists to Coon Creek peat experimental plots, commercial vegetable growers (peat soil), early potato section at Osseo, market garden area at Brooklyn Center.
Thursday P. M.	Field trip continued to Minnesota Fruit Breeding Farm (accompanied by plant pathologists and horticulturists).
Friday, Saturday	A field trip to Itasca State Park, the Cloquet Forest Experiment Station, and North Shore of Lake Superior, joint with Plant Science Group.

Titles of papers for the symposium on dormancy, after ripening, and germination of seeds, and also papers of general interest, should be sent to Ralph W. Lorenz, Secretary of Minnesota Section, Division of Forestry, University Farm, St. Paul, Minnesota.

The Lowry Hotel in St. Paul has given proper assurance that out-of-town guests will be given every consideration and adequate accommodations if they wish to make it their lodging headquarters. Please make reservations early.

St. Louis Meeting.—The twelfth annual meeting of the American Society of Plant Physiologists will be held late in December, 1935, at St. Louis. It is not too early to begin planning for this important series of meetings. Members of the Society can aid the program committee and other officers responsible for the arrangements by prompt response to calls for service. If symposia are contemplated early decisions as to topics and invitation papers would be desirable in order to permit more deliberate preparation by participants. The quality of our meetings can be much improved by careful planning and choice of material. Programs frequently list too many unrelated papers, and so many papers are offered that the program committee should no longer feel obliged to accommodate all offerings. Each meeting affords an opportunity to get out of ruts or fixed grooves and to improve the technique of meetings in general.

Minnesota Section.—The Minnesota Section of the Society has held meetings once each month during the year, with an attendance of 15 to 30 at each meeting. The speakers who have addressed the section during the year and the topics under discussion are as follows:

Dr. J. F. McClendon, Physiology in Japan and China.
Mr. F. B. Chandler, Winter injury in apple trees.
Mr. R. E. Oltman, Research on chlorophyll and photosynthesis.
Dr. G. O. Burr, Current physiological research in Europe.

The officers of the Minnesota Section for 1935 are Dr. R. H. Landon, chairman, and Mr. R. W. Lorenz, secretary. Dr. Landon and Dr. Harvey are also taking care of the program and entertainment arrangements for the summer meetings. Those who several years ago attended a previous summer meeting of the Society under the auspices of the Minnesota Section will surely anticipate with much pleasure the June meetings.

Editorial Committee.—As was announced in January, the editorial committee is to be reorganized. The executive committee has now arranged the procedure for rotational retirement and annual appointment of memmers of the editorial board of Plant Physiology. The present members of the board have been appointed with retirement dates as follows: C. A. Shull, editor-in-chief, 1939; B. E. Livingston, 1938; F. E. Lloyd, 1937; C. B. Lipman, 1936; C. R. Ball, 1935. As each member retires, a successor will be appointed for a 5-year term. This arrangement provides a degree

of permanence and stability, but permits a slow change in personnel if the executive committee thinks it desirable. The addition of elective members chosen by the society at large waits upon constitutional authority to make such enlargement of the board.

Finance Committee.—The finance committee has been placed upon a rotational basis of appointment. As now constituted the finance committee consists of three members: C. A. SHULL, chairman, appointed for 3 years; A. E. MURNEEK, 2 years; W. F. LOEHWING, 1 year.

International Botanical Congress Delegate.—The American Society of Plant Physiologists will be officially represented at the sixth International Botanical Congress at Amsterdam in September, 1935, by Dr. WALTER F. LOEHWING, University of Iowa.

Chemical Methods Committee.—The committee on chemical methods has been enlarged by the appointment of Dr. H. R. KRAYBILL, Purdue University, to membership on the committee. The committee will continue to serve as a clearing house of information concerning methods of analysis most useful to plant physiologists.

Chemical Methods Committee Report.—The first supplementary report of the committee on chemical methods appears in this number of PLANT PHYSIOLOGY. The chairman of the committee, Dr. W. E. TOTTINGHAM, Department of Agricultural Chemistry, University of Wisconsin, Madison, Wisconsin, will have a supply of the reprints of this report bound together as a single reprint. These will be available at a price to be determined by their cost. Announcement of the price will be made in the July number of PLANT PHYSIOLOGY. When it is possible to do so, members should send in club orders for the number needed locally by members and students. This will materially decrease the cost of distribution.

International Address List.—A third revised edition of the international address list of plant physiologists has been authorized by the executive committee. It is to be published as Bulletin no. 9 of the American Society of Plant Physiologists, and it is hoped it will be ready for distribution to the members this fall. This address list has been issued at about 5-year intervals, and is a very useful bulletin. As in the case of the first two editions, this one will be compiled and edited by Dr. R. B. HARVEY, University of Minnesota.

Low Temperature Relations Bibliography.—For many years Dr. R. B. HARVEY has been engaged in compiling a fairly complete list of the publications dealing with the low temperature relations of plants. This list has now been mimeoprinted by the Burgess Publishing Co., Minneapolis,

with the title "An annotated bibliography of the low temperature relations of plants." There are 195 pages of citations, two columns to the page, and a listing of more than 3,400 items. There are very brief annotations when the titles fail to indicate the nature of the contents of the papers. A subject index occupies 28 pages.

The bibliography represents a very large and unselfish expenditure of time and energy. It will be a most useful work to those who are engaged in investigating any problem involving the effects of low temperatures on plant behavior. As science becomes more complex, and scientists more numerous and more prolific, such bibliographies become more and more necessary to progress in research. The author of this work and his aids deserve praise for having made available this valuable bibliography. The price of the volume in flexible binding is $4, and orders may be sent direct to the publishers.

Biochemical Laboratory Methods.—A second edition of Dr. C. A. Morrow's Biochemical Laboratory Methods has been prepared by Dr. W. M. Sandstrom. The chapters have been reorganized and modified to fit the work to serve as a laboratory manual to go along with Gortner's Outlines of Biochemistry. The book is somewhat reduced in size, from 350 to 319 pages, owing to omission of some material more adequately supplied in the Outlines. It is a very helpful laboratory manual. The publishers, John Wiley and Sons, quote the price as $3.75 per copy.

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ACCUMULATION OF BORON BY RECIPROCALLY GRAFTED PLANTS

FRANK M. EATON AND GEORGE Y. BLAIR[1]

(WITH THREE FIGURES)

Introduction

The experimental data presented in this paper show that the amount of boron accumulated by plants may be materially influenced by the rootstock. The boron relations of agricultural plants have been found to be diverse and the characteristics of boron accumulation are in some measure differentiated from the accumulation characteristics of other elements. Certain facts in this regard, illustrated in the following three paragraphs and more extensively presented elsewhere (1), provide in a measure a background for the experimental results here to be considered.

An outstanding variability exists in the extent to which different plants take up boron. The boron concentrations resulting in entire plants, expressed in parts per million on the basis of dry weights, when grown in sand cultures maintained with a nutrient solution containing 5 p.p.m. of the element, were as follows: Navy beans 648, lima beans 515, wheat 453, pumpkin 291, lettuce 261, turnips 245, Canadian field peas 207, tomatoes 159, alfalfa 139, Acala cotton 123, asparagus 120, and carrots 69.

The distribution of boron throughout a plant is not uniform; it tends to accumulate in some parts more than in others. The leaves of rooted lemon cuttings grown in the cultures just mentioned contained 1232 p.p.m. of boron and the combined stems and roots 54 p.p.m. Young elms had 943 p.p.m. in their leaves and 22 p.p.m. in their stems; corn had 60 p.p.m. in the combined leaves, stalks, and roots and 16 p.p.m. in the grain; roses had 220 p.p.m. in their leaves and 20 p.p.m. in their stems. Twigs of boron-injured prune trees in an orchard planting contained 176 p.p.m. of boron in their leaves,

[1] Special acknowledgment is made of the indispensable analytical work carried out by V. P. SOKOLOFF and L. V. WILCOX.

412 p.p.m. in the bark, and 171 p.p.m. in the wood. Twigs of slightly injured lemon trees on the other hand contained 410 p.p.m. in their leaves, 42 p.p.m. in the bark, and but 9 p.p.m. in the wood. As shown by SCOFIELD and WILCOX (7), orange trees with 854 p.p.m. of boron in their leaves contained but 40 p.p.m. in the dried fruits.

Within particular plant parts differences in accumulation are likewise found. A sample of injured lemon leaves showing marginal burning and the characteristic yellowing of the leaf tissue farther removed from the veins had 47 p.p.m. of boron in the midveins and petioles, 438 p.p.m. in the green portion of the leaf laminae, 1060 p.p.m. in the yellow portions, and 1722 p.p.m. in the dead margins and apices. The bark at the enlarged nodes of the boron-injured prune twigs just referred to contained more boron than the bark of the internodes.

The facts that in most plants boron tends to accumulate in leaves, and in these at points farthest removed from the water-conducting tissue, suggest that it is carried to the leaves by the transpiration stream. The same facts also imply that boron as taken up and as moved to the leaves (probably as an inorganic radical) is highly mobile, but upon reaching the leaves much of it is converted into compounds that are not very mobile. These less mobile compounds probably represent the result of reactions with organic constituents in the leaves.

We should expect the mobility of boron compounds or the equilibria between mobile and non-mobile compounds as they exist in the leaves or elsewhere in the plant to affect not only the distribution of boron in the various parts of the plants, but also the rate of uptake from the soil or culture solution. Boron moved to the absorbing roots, as by transport through the phloem, would be expected to depress absorption rates; whereas very low concentrations in the absorbing root cells should tend to favor increased absorption rates.

It is reasonable to assume that mobility (4) is a factor that may have a marked effect upon absorption rates, but absorption rates as they relate to concentration potentials across absorbing membranes must likewise be considered in the light of membrane characteristics. We cannot assume that the absorbing root cells of all plants tend to maintain like concentration potentials. The data submitted in this paper show that the amount of boron accumulated by plants can be altered by substituting rootstocks. While no record has been found in the literature of an effect of rootstock on the accumulation of any electrolyte in the above-ground portions of plants, consideration has nevertheless been given to this possibility. SWINGLE, ROBINSON, and MAY (9), having observed in greenhouse tests in Washington in 1928 that *Severinia buxifolia* (a citrus relative) was more tolerant to boron than the several citrus varieties compared with it, suggested that the plant

for this reason might provide a promising rootstock for commercial citrus in areas supplied with boron-contaminated irrigation water.

Since a grafted plant may be a combination of two varieties of the same species, of two species, or even of two genera, it is surprising that so little work dealing with the effects of rootstocks upon absorption of nutrient or toxic ions should have been conducted or recorded. Only two reports of such studies have been found. Roach (6) has reported the spectroscopic examination of the wood of one of two apple stocks to have shown molybdenum to be present. But this element was not found in the wood of the one scion variety grafted to these stocks. No spectroscopic evidence was obtained that the two rootstocks were different in their absorption of other elements or that the rootstock affected the accumulation of electrolytes in the scion variety. Haas and Halma (2) have shown that differences in the solubility but not in the total amount of magnesium of the bark of citrus rootstocks resulted when field-grown trees had different scions. Different rootstocks did not give rise to differences in the solubility or quantity of magnesium in the bark of the scion. The concentration or solubility of calcium was not affected either in the stock or in the scion by the several combinations studied.

The primary purpose of the studies here reported was to determine whether boron accumulation is primarily conditioned on root characteristics or on characteristics of or reactions in the above-ground organs. In electing to use reciprocally grafted plants as a method of experimental approach to this question, it was recognized as essential that the plants employed should be well contrasted with respect to boron uptake. The Jerusalem artichoke and the sunflower were selected as one plant pair and the lemon and Chinese box orange as another.

The Jerusalem artichoke (*Helianthus tuberosus*) is sensitive to boron, being severely injured when boron is present in culture solutions in concentrations as low as 5 p.p.m. The sunflower (*Helianthus annuus*), on the other hand, is only moderately tolerant to boron; its growth is not greatly reduced by culture solutions containing as much as 15 p.p.m. The artichoke, when grown beside the sunflower in the same culture solution, accumulates in its leaves higher concentrations of boron than does the sunflower. The two plants, which may be readily intergrafted, are different in an important physiological respect; inulin is the storage carbohydrate of the artichoke, whereas starch is the storage form in the sunflower.

The lemon (*Citrus limonia* Osbeck) is highly sensitive to boron; in fact few plants are thought to be more sensitive, and it accumulates much boron in its leaves. The Chinese box orange, *Severinia buxifolia,* on the other hand, in confirmation of the observation of Swingle, Robinson, and May, is relatively tolerant to boron. *Severinia* has been found to accumulate

much less boron in its foliage than does the lemon. The writers are indebted to SWINGLE, ROBINSON, and MAY for supplying the *Severinia* and reciprocally grafted lemon and *Severinia* plants used in these experiments.

Experimental methods

SUNFLOWERS AND ARTICHOKES

The sunflowers and artichokes on their own roots and the reciprocally grafted sunflowers and artichokes were grown out of doors in large sand culture vessels during the summers of 1932 and 1933 at Riverside, California. The base culture solution had the following composition: $Ca(NO_3)_2$, $MgSO_4$, KH_2PO_4, in concentrations of 4, 2, and 2 millimoles per liter respectively. In addition there were added $MnCl_2$ to give 0.5 p.p.m. of manganese, 1 cc. per liter of a 5 per cent. solution of iron tartrate, and boric acid in sufficient amounts to give the concentrations of boron subsequently indicated. This solution was applied daily in sufficient excess to produce 50 per cent. or more of drainage from the sand culture vessels.

In both years the plants were all grown together in a single vessel, thus assuring that they were subjected to like concentrations of the boron supplied.

In 1932 the seeds and tubers were planted on April 12 and the artichokes and sunflowers reciprocally grafted by the approach method on June 10. From time of planting until July 8 the nutrient solution contained 1 p.p.m. of boron. The same nutrient solution but with 5 p.p.m. of boron was used from July 8 until the plants were harvested on August 19. In other words, these plants received the high boron solution for 42 days at the end of which period some of the older artichoke leaves had been killed.

In 1933 the sunflower seeds were planted on April 11 and the artichoke tubers on May 14. The grafting was done during the last week of June. These plants were grown with 1 p.p.m. of boron in the nutrient solution until September 1 and thereafter until harvested on October 1 with a 5 p.p.m. solution. By September 1 all branches of both sunflowers and artichokes were terminated by a flower or flower bud; no new leaves were formed therefore by the 1933 plants during the high boron treatment. In 1932 new leaves developed after the high boron treatment had been started.

In 1932 separate samples were taken of old and young leaves but not all leaves on any plant were included. In 1933 each leaf sample included all of the leaves, either sunflower or artichoke, on a particular plant. Enough boron may accumulate in a mature Jerusalem artichoke leaf of a plant growing in a 1 p.p.m. boron solution to cause some injury, and while some boron will likewise accumulate in sunflower leaves there will be no visible evidence of injury.

In 1932 the stem samples were of 9-inch segments above and below the point of the graft on the grafted plants and above and below a comparable point on the ungrafted plants. The 1933 stem samples were of entire stems divided only as to artichoke or sunflower.

Lemons and *Severinia*

The base nutrient solution used for the lemon and orange experiments was the same as that used for the artichoke and sunflower experiments but the boron concentrations of the solutions as applied were 2, 4, and 6 p.p.m. These concentrations were used for 22 months before the samples of mature leaves were collected for analysis, February 2, 1934.

The lemons on their own roots were three years old from cuttings. The oranges on their own roots and the reciprocally grafted lemons and oranges were 3 and 4 years old and were also from cuttings.

For the most part the different plants were grown in separate 20-gallon sand culture cans, although some were paired in the same cans (table II). Except when the plants were in pairs in the same containers, it is not implied that the average boron concentrations as presented to the roots were identical for all plants of a series. Under Riverside climatic conditions the concentrations of boron in culture solutions such as were here used increase as the volumes are decreased by transpiration. The transpiration losses from the different sand-culture cans were different owing to differences in plant sizes. The 2, 4, and 6 p.p.m. boron concentrations therefore should be looked upon as minimum concentrations. The culture solutions were applied daily in quantities roughly in proportion to plant sizes and always in sufficient amount to produce copious drainage. Only mature leaves were selected for analysis. The average age of leaves making up each of the samples undoubtedly varied a little. This variation, like the possible variation in the concentration of boron in the culture solutions held by the sand in cans receiving like treatments, would affect to some extent the quantity of boron accumulated.

Experimental results

Sunflowers and artichokes

The results of the 1932 experiment are reported in table I. The concentrations of boron found in the samples of the sunflower leaves were 50 per cent. greater when this plant was grown on artichoke roots than when grown on its own roots; for the older leaves the values were 1470 and 936 p.p.m. respectively. Conversely, when the artichoke was grown on its own roots the older leaves were found to contain 1520 p.p.m. of boron whereas when on sunflower roots the older artichoke leaves contained but 711 p.p.m. of boron.

TABLE I

BORON IN LEAVES AND STEMS OF SUNFLOWERS AND ARTICHOKES WHEN ON THEIR OWN ROOTS AND WHEN RECIPROCALLY GRAFTED; 1932 EXPERIMENT

PLANT	BORON (DRY WEIGHT)
Sunflower on own roots	*p.p.m.*
Older leaves	936
Younger leaves	494
Stems: upper segment	30
Stems: lower segment	24
Artichoke on own roots	
Older leaves	1520
Younger leaves	1224
Stems: upper segment	63
Stems: lower segment	50
Sunflower on artichoke roots	
Older leaves	1470
Younger leaves	1128
Stems: sunflower segment	40
Stems: artichoke segment	43
Artichoke on sunflower roots	
Older leaves	711
Younger leaves	414
Stems: artichoke segment	21
Stems: sunflower segment	26

The sunflower leaves showed only mild injury when the plant was on its own roots but the injury was marked when on the artichoke roots (fig. 1). The artichoke leaves were severely injured when the plant was on its own roots; while on sunflower roots the injury was perhaps 60 per cent. as severe.

The results of the 1933 trials are shown diagrammatically in figure 2. The methods were different from those of 1932 in that the plants were not subjected to the high boron concentration until they had flowered, and then for only a 30-day period during which no new leaves were formed. Also in 1933 branches of the rootstock variety were allowed to develop below the grafts. All leaves of a species on each individual plant were combined as single samples irrespective of age.

As shown in figure 2, the concentrations of boron in the leaves of two sunflower plants on their own roots were 575 and 684 p.p.m., whereas sunflower leaves on plants with artichoke roots contained 1026 and 954 p.p.m. of boron. Conversely, the leaves of artichoke plants on their own roots contained 893 and 879 p.p.m. of boron, whereas on sunflower roots

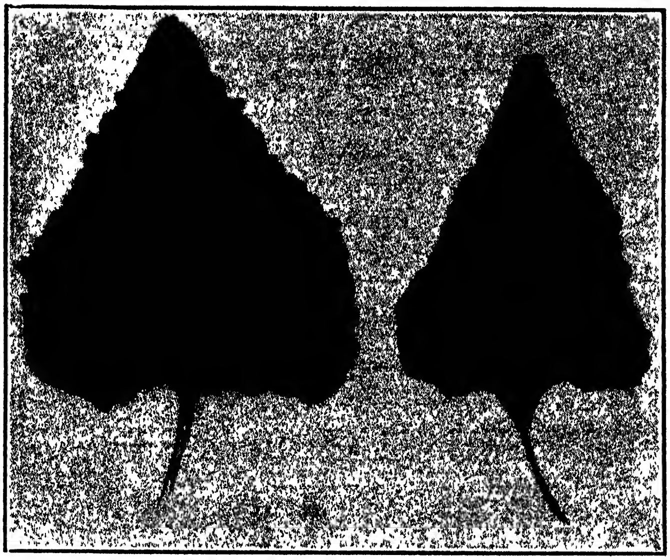

FIG. 1. Sunflower leaves of like ages: leaf on left is from a plant on its own roots and shows only a little boron injury along its margin; leaf on right is from a plant on artichoke roots and is severely injured.

artichoke leaves contained 663, 690, and 644 p.p.m. of boron. The anomaly presented, for example, by the high accumulation of boron in basal sunflower branches of plants with artichoke tops will be dealt with in the discussion.

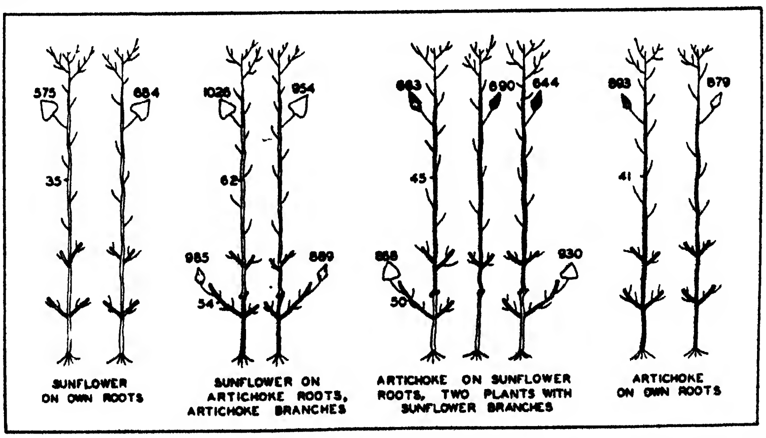

FIG. 2. Sunflowers and artichokes of 1933 experiment: boron concentrations, expressed as p.p.m. of dry weights, represent samples of all leaves and of entire stems of each plant, separated (as designated) only as to species.

LEMONS AND *SEVERINIA*

The effects of reciprocally grafting lemons and *Severinia buxifolia* on the accumulation of boron in the leaves are set forth in table II.

TABLE II

CONCENTRATIONS OF BORON IN LEMON AND *SEVERINIA* LEAVES (DRY WEIGHT) AFTER GROWING FOR 22 MONTHS IN SAND CULTURES SUPPLIED WITH NUTRIENT SOLUTION CONTAINING 2, 4, AND 6 P.P.M. OF BORON

PLANT	BORON IN CULTURE SOLUTION		
	2	4	6
	p.p.m.	*p.p.m.*	*p.p.m.*
Eureka lemon on own roots			
Can: 2B–12 4B–13	637	1065	Dead
2B–13	724		
Eureka lemon on box orange roots			
Can: 2B–10 4B–10 6B10	223	283	517
Lisbon lemon on box orange roots			
Can: 2B–13 4B–13 6B–12	253	397	457
Box orange on Eureka lemon roots			
Can: 2B–10 4B–10	528	877	
Box orange on own roots			
Can: 6B–11–1			390

These results are similar to those obtained with sunflowers and artichokes but are perhaps even more outstanding. In terms of the concentrations of boron accumulated, the lemon and box orange are more sharply contrasted than the sunflower and the artichoke.

Lemon on box orange roots had about one-third as much boron in their leaves as when grown on their own roots. The data indicate that the boron concentrations in the box orange leaves are increased threefold when the plant is grown on lemon roots. There were no box orange plants on their own roots in the 2 and 4 p.p.m. cans, but the plant in 6 p.p.m. on its own roots had 390 p.p.m. whereas the box orange on lemon roots in 4 p.p.m. had 877 p.p.m. of boron in its leaves. Lemon leaves on their own roots were more severely injured than when on the box orange roots (fig. 3).

Both Eureka and Lisbon lemons were used in these tests. We believe that the reactions of the two varieties to boron may be considered identical since no differences were found in their boron accumulation characteristics.

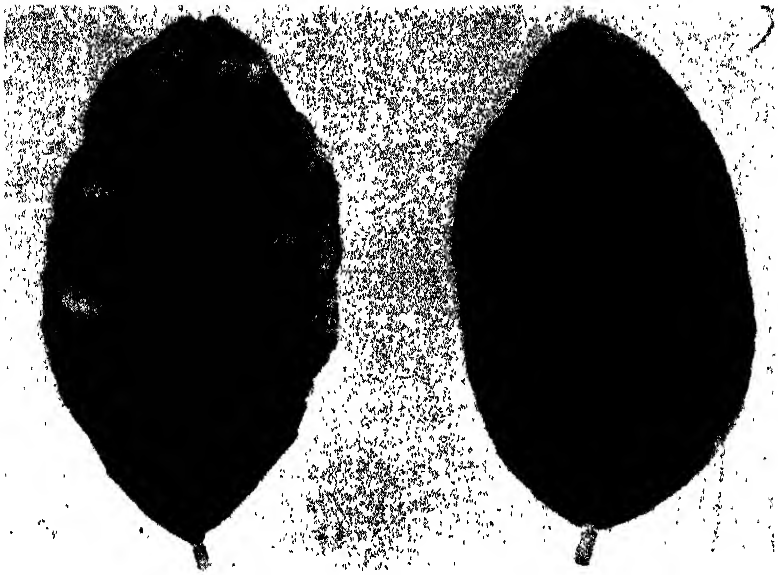

FIG. 3. Lemon leaves: leaf on left is from a plant on its own roots and shows marked boron injury; leaf on right is from a plant on *Severinia* roots and shows little boron injury.

Discussion

The data presented show that the quantities of boron accumulating within plants may be affected in an outstanding way by the character of the rootstock. The boron relations of plants, however, are known to be broader and more complex than these experiments would suggest. That the character of the rootstock does not stand alone as a factor determining boron absorption, accumulation, and injury may be illustrated by the behavior of Payne and Eureka walnuts on black walnut roots.

Under field conditions it has been found that the Payne walnut shows less severe leaf injury than the Eureka variety when the two are grown on black walnut (*Juglans hindsii* Jepson) roots in the same grove. Leaves of the Payne variety have likewise shown less injury and have uniformly contained less boron than leaves of the Eureka variety when the two were grown on black walnut roots side by side in sand cultures (table III).

Tolerance to boron is only in part a matter of non-accumulation. Marked differences exist in the concentrations of boron found in the leaves of different species showing a similar severity of injury. The Navel orange accumulates perhaps slightly more boron in its leaves than does the lemon when both are grown on like rootstocks under comparable conditions, and yet the injury shown by lemons greatly exceeds that shown by oranges. Oranges

TABLE III

BORON IN LEAVES (DRY WEIGHT) OF PAYNE AND EUREKA WALNUTS ON BLACK WALNUT ROOTS WHEN GROWN IN PAIRS IN SAME CULTURES. CULTURE "O" CONTAINED A TRACE OF BORON FROM IMPURITIES OF THE CHEMICALS AND SAND. CONCENTRATIONS OF BORON IN OTHER BEDS WERE MAINTAINED AS INDICATED. LEAVES WERE COLLECTED AT END OF FIRST GROWTH SEASON AFTER TRANSPLANTING TREES WITH YEAR-OLD BUDS FROM FIELD

VARIETY	BORRON IN CULTURE SOLUTION				
	"O"	1	3	6	9
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Payne	46	162	468	728	1280
Eureka	81	260	579	902	0*

* No leaves retained; all were severely injured and abscissed.

will withstand possibly twice as much boron in the soil or culture solution before injury comparable with that of the lemon is shown. It is most reasonable to suppose that differences in the severity of injury that results from like concentrations of boron in the leaves of different plants are related. at least in part, to the nature of the boron compounds present. Some of these compounds may have a far greater toxicity than others. The first evidence of injury is manifest by a reduction in the amount of chlorophyll in the leaf; *i.e.*, portions of the leaves turn yellow. This reduction is associated with boron accumulation but as yet we are unable to conclude whether the effect is a direct or an indirect one.

Boron is known to be reactive with a number of organic compounds normally occurring in plants and to form with them complex molecules. Boric acid combines readily with ethyl alcohol to form tri-ethyl borate, $(C_2H_5)_3BO_3$, and with methyl alcohol to form tri-methyl borate, $(CH_3)_3BO_3$. Boron also unites with various higher alcohols (glycerol, erythritol, mannitol, sorbitol, dulcitol) and with certain of the sugars to form compounds of uncertain character but probably with ester-like linkages. Analytical use is made of the reaction with mannitol which, when added to a solution of boric acid, liberates one H ion for each molecule of boric acid present. SHKOL'NIK (8) cites data by INOZEMCEV showing that boron accelerates the gelatinization of gelatin, increases the viscosity and decreases the distention. He submits his own data to show that boron exerts an effect on the permeability of roots. Without boron in culture solutions the absorption of PO_4, NO_3, and Ca by flax and *Vicia faba* was greatly increased over control plants supplied with boron, whereas K absorption was depressed. These relationships indicate that boron has a marked effect on the permeability of the plant protoplasm and that it may combine with proteins or their derivatives, but the reactions between boron and the amino acids or proteins apparently have not been investigated.

Only a little is known about the character of organic compounds of boron, and while there may be many of them, they have received scant attention. Attempts to isolate, identify, or measure the quantities of such compounds in plant material or to trace their movement would be of uncertain outcome and scarcely warranted at the present time. The total-boron analyses presented in the introduction provide evidence that boron, after reaching the leaves, forms compounds that are not readily translocated. The same data indicate that in different plants there are differences in the extent of movement. These facts favor the complex organic-molecule interpretation of low mobility.

In the sunflower-artichoke experiments it was found that the leaves of basal sunflower branches of plants with sunflower roots and artichoke tops contained 868 and 930 p.p.m. of boron whereas sunflower leaves on plants without the artichoke top contained 575 and 684 p.p.m. of boron. These observations can be explained if we assume either that the boron of the artichoke is more mobile than that of the sunflower or that the ratio of mobile boron to non-mobile boron is higher in the artichoke than in the sunflower. On either basis a movement of boron from artichoke leaves to sunflower leaves should occur with a resultant accumulation in the sunflower leaves of boron converted into a less mobile form.

It has been suggested that differences in transpiration rates might account for the marked variability in the boron absorption characteristics of different plants. Without attempting to minimize the possible importance of this factor, it is nevertheless desirable to point out that the total boron taken up by plants when considered with reference to the total transpiration shows the ratios of water absorbed to boron absorbed to be high. Transpiration, for example, would fail to account for the threefold effect on boron accumulation that resulted when the lemon was grown on *Severinia* roots or when *Severinia* was grown on lemon roots.

On the basis of the foregoing, two theories may be advanced as to why plants show differences in the quantity of boron absorbed. The first recognizes, as between plants, differences in the characteristics of the protoplasts of the absorbing roots that affect the equilibria between external and internal boron concentrations and therefore the rate of boron uptake. The second recognizes, as between plants, differences in the forms and in the equilibria concentrations of the non-mobile and mobile boron of the leaves and other organs that would be expected in turn to affect boron concentrations in the root cells and accordingly the rate of uptake. Low concentrations of mobile boron by the latter theory would favor low concentrations in the root cells and increased rates of uptake and relatively high concentrations of total boron in the leaves. The many facts which have been accumulated with regard to plant reactions to boron can best be explained by assuming that

both these theories are essentially valid and that both may be operative in one and the same plant at the same time, acting either in the same or in opposite directions to varying degrees.

The researches of OSTERHOUT (5) and others demonstrate that single cells may show outstanding differences in their intake equilibria. But as HOAGLAND and DAVIS (3) emphasize in their study of the intake of electrolytes by *Nitella,* the mineral nutrition of plants involves much more than a question of cell permeability. That higher plants grown on the same substratum differ in the quantity and proportion of ions they absorb needs no confirmation, for differences are the rule rather than the exception. The work of MASON and MASKELL (4) on the transport of calcium, phosphorus, nitrogen, and potassium through the phloem can leave little doubt regarding either the downward movement of several of these constituents from the leaves to the roots or their reappearance in the transpiration stream. Elements returned to the root cells through the phloem, as they believe to be the case, must almost unquestionably affect absorption rates. MASON and MASKELL's deductions as to the mobility of various forms of nitrogen and carbohydrates conform with the conditions here deduced with respect to the mobility of boron.

The obvious conclusion to be drawn from the boron experiments is that both root-cell permeability and the mobility of the boron compounds occurring in plants affect uptake and accumulation. Whether or not valuable field application can be made of these possibilities is a question that cannot now be answered. The selection of rootstocks possessing desirable absorption characteristics can constitute only a part of inquiries directed toward practical ends, since rootstocks are to be chosen also on the basis of such factors as disease resistance, vigor, and compatibility.

The foregoing discussion naturally leads to the more general question of whether the rootstock effect here observed is limited to boron. It is recognized that the reaction of plants to high concentrations of boron is in some respects outstanding and in a measure differentiated from plant reactions to other inorganic constituents of the soil. Nevertheless there seem to be no grounds for assuming that the rootstock effect may not be a common one. A question is likewise presented as to whether certain rootstocks might be more profitably used than others for the culture of orchard crops on soils deficient in one or more nutritive elements. If the effect with respect to certain other ions is a common one, then it is not at all unlikely that a measure of advantage has already been taken of it in the choice of rootstocks in older orchard sections, even though the selections may have been made on the basis of observed suitability by a trial and error process rather than through any recognition of cause.

For the purpose of determining whether sunflowers and artichokes or Chinese box oranges and lemons were different with respect to the accumulation of calcium, magnesium, sodium, potassium, or sulphate, analyses were made of the leaves of these plants as grown on their own roots. These analyses did not show differences sufficiently well marked with respect to any of the determined ions to offer promise of significant differences in the reciprocally grafted plants.

Summary

1. There is an outstanding variability in the extent to which different plants take up boron. The boron accumulated by plants is not uniformly distributed in the different organs or parts but tends to accumulate in some parts to a much greater extent than in others. Within particular plant parts the accumulation of boron is by no means uniform; 30 times as high a concentration of boron has been found in the margins of lemon leaves as in the midveins and petioles. It is believed that boron is carried to the leaves by the transpiration stream as an inorganic radical but that on reaching the leaves much of it combines with organic compounds that are not very mobile.

2. The boron concentrations accumulating in the leaves of the scion were directly influenced by the rootstocks upon which they were grown. The scion leaf concentrations were reduced if grafted to varieties normally accumulating lesser concentrations and increased if grafted to varieties normally accumulating higher concentrations. Concentrations of boron in scion leaves tended to approach those normal to the rootstock variety. Reciprocally grafted sunflowers and Jerusalem artichokes were used in one series of experiments and lemons and Chinese box oranges in another.

3. Boron absorption is not wholly contingent upon the rootstock employed. Eureka walnuts accumulate more boron in their leaves than the Payne variety when both are grown in the same cultures on black walnut roots. Different species accumulating comparable concentrations of boron in their leaves may show marked differences in severity of injury.

4. With other conditions equal, the rate of boron absorption is determined by (1) the characteristics of the absorbing root cells, and (2) the nature of the boron compounds in a plant and the equilibria between mobile and non-mobile forms. The mobility of boron in the plant or the proportions of mobile and non-mobile boron would be expected to affect not only the distribution of boron in different parts of the plant but also the concentration of boron in the root cells and accordingly the rate of uptake.

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SALT TOLERANCE OF *RUPPIA MARITIMA* IN LAKES OF HIGH MAGNESIUM SULPHATE CONTENT[1]

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Introduction

The ability of halophytes to develop under conditions which would mean death to most vegetation is well known. Salt marsh plants, growing where sodium chloride is the chief constituent of the soil solution and where salt concentrations usually vary from 1 to 6 per cent., are the most common halophytes. Much less numerous than the salt marshes are the lakes whose chief constituent is magnesium sulphate, a compound toxic to many plants in any but very dilute concentrations. Although the plants of the two types of lakes are to some extent the same, very little work has been done on those of the magnesium sulphate lakes. In view of the common toxicity of magnesium salts to plants and the scarcity of information concerning the physiological relations of halophytes, it has seemed worth while to investigate the vegetation of certain lakes lying in north central Washington and adjacent British Columbia which are unusual in having a very high proportion of magnesium sulphate in the salt content and in supporting seed plants at salt concentrations as high as 25 or 26 per cent. Studies were made on three lakes lying in the eastern foothills of the Cascade Mountains in the northern part of Okanogan County, Washington, and British Columbia, in one of which *Ruppia maritima* L. grows abundantly, completely submerged in the salty water of the lake.

The first of these, known locally as Epsom Lake and designated in this paper as no. 1, lies about 4 miles northwest of the town of Oroville, in northern Washington, about midway between Lake Osoyoos and the Similkameen River. The geology of the region was described by JENKINS (**10**). ST.JOHN and COURTNEY (**35**) listed the phanerogams growing in the lake and on the walls of the surrounding basin. They report the banks to be of practically pure salt, in which the plants were apparently rooted. Analyses of salts taken from the surface of the water gave a content of 99.64 per cent. of $MgSO_4$.

The lake lies at an elevation of about 2000 feet, with walls of the basin reaching several hundred feet higher. The lake itself, which drains an area of about one square mile, is, when full, approximately four acres in extent and 30 feet deep. There is no outlet and drainage waters remain until evaporated during the dry summer season, when the lake is reduced to a series of small

[1] Contribution no. 39 from the Botany Department of the State College of Washington.

pools and the bed becomes white and crystalline in appearance. The surrounding vegetation is sparse.

The second lake studied, designated no. 2, lies several miles north of no. 1, in British Columbia, on a ridge of Kruger Mountain, about 8 miles from the town of Osoyoos. It is known locally as Spotted Lake, owing probably to its spotted appearance when summer evaporation has reduced it to a series of round pools. Like no. 1, Spotted Lake is the drainage basin for the surrounding hills and has no outlet. It is considerably larger than no. 1, being about one-half mile long and 300 yards wide. The elevation is somewhat greater than that of lake no. 1 but the walls of the basin are not so steep. Vegetation is even more sparse than around lake no. 1 and no sign of plant life is found in the waters of the lake except a few algae. The lake contracts to a white bed with round pools of saturated water after a dry period (**10**).

Lake no. 3 lies a few miles to the south of no. 2 and at a greater elevation on Kruger Mountain. It is much larger and considerably deeper than the others. Although it is much less concentrated than the others there is no evidence of plant or animal life and no growth of plants for some distance from the water's edge. The soil of the banks is colored and sandy, and is not so white and crystalline in appearance as that of the other two lakes.

Literature review

Until recently work on halophytes has been concerned chiefly with their ecological relations. Schimper (**34**) described halophytes as those plants which are capable, owing to xeromorphic adaptations of physiological and anatomical characters, of living under conditions of physiological dryness where water, although present, is not readily available to the plant. Fitting (**7**) found halophytes able to develop osmotic pressures of sufficient value to overcome the resistance to absorption of greatly concentrated soil solutions. Delf (**4**) found the transpiration rates of *Salicornia annua* and *Suaeda maritima* comparable to mesophytes such as *Vicia faba,* the halophytes having no stomatal modifications to reduce transpiration. Yapp (**40**) described the xeromorphic character of *Spiraea ulmaria,* showing the development of characters usual in xerophytes. Apparently halophytism may be expressed in either physiological or morphological adaptations or both.

Osterhout (**15**), in working with marine plants, found that each of the salts of sea water is poisonous when alone but that the mixture, as found in sea water, gives a "physiologically balanced" solution which the plant is capable of using. The idea of balance of solution was soon extended to include fresh water and terrestrial plants (**16**). Since then extensive investigations by Osterhout (**17, 18, 19, 20, 21, 22, 23, 24, 25**), True and

BARTLETT (**37**, **38**), RABER (**27**, **28**, **29**, **30**, **31**), SCARTH (**33**), and many others have laid the basis for the theory of the antagonism of ions, have secured much information concerning the antagonistic relations of various ions, and have added greatly to our knowledge of the effects of acids, alkalies, and anions and kations of different valences on the permeability of plant membranes. OSTERHOUT (**26**) and HOAGLAND and DAVIS (**9**) have given analyses of the cell sap of *Valonia* and *Nitella* respectively, as compared with the water in which they were growing.

Recently consideration has been given to the salt requirements of salt marsh plants. Analyses made by ZELLNER (**41**) of *Salicornia herbacea, Suaeda salsa, Scorzonera parviflora, Plantago maritima, Aster tripolium,* and *Erythraea tinariafolia* growing on soils containing about 3 per cent. of soluble salts showed all of the plants to contain large amounts of Na, Cl, and SO_4. Over 20 per cent. of the dry weight was found to be ash in *S. herbacea* and *S. salsa.* These results are in accord with those of KILLIAN (**11**), who concluded, after studying plants growing in soils rich in salt, that NaCl may be in even greater concentration in the plants than in the soil, and that different species growing in the same soil may show great variation in the salt accumulated in the plant. MONTFORT and BRANDRUP (**12**, **13**, **14**) concluded that the resistance of plants to high salt content varies at different stages of development of the plant as well as with different concentrations of salt. The pH of the soil solution plays a minor rôle, but the proportion of Cl and SO_4 is important in determining plant distribution. RIKHTER (**32**) likewise found balance important, in that plants can tolerate much greater concentrations of balanced solutions than of pure salts. He groups plants able to tolerate high content of salt into two types with regard to the method of developing the internal osmotic pressures necessary to overcome the external pressures. Such plants as *Helianthus, Triticum, Amaranthus,* and *Artemisia* increase their own supply of osmotically active materials, while halophytes such as *Salsola, Suaeda,* and *Salicornia* can accumulate salts from the outside medium without harm to themselves. Plants of the first group are characterized by a high impermeability of the roots, so that the power to exclude the salts determines the limit of concentration which the plants can endure. These workers were interested in plants growing in salt concentrations close to that of sea water. BRIGHENTI (**2**) describes salt marshes of the province of Ferrara, Italy, near the Adriatic Sea. These have a depth of 50–90 cm. In summer the NaCl reaches 41 per cent. concentration, although this is diluted in the winter by rain and by the Po di Volano. No phanerogams grow in the marshes but some diatoms are found, and the algae *Ulva latissima* and *Chaetomorpha crassa* are abundant.

FLOWERS (8) describes the vegetation of the Great Salt Lake region, listing the plant associations of the lake, the dunes, salt marshes, etc., and giving ecological data including the salt content of soils and soil waters. He compiles tables of analyses made of Great Salt Lake water at different periods from 1877 to 1910. The salt content varied from 13.79 to 27.72 per cent. during the period. NaCl is the principal constituent. The Cl analyses of the total salt vary from 55.1 to 56.54 per cent. Na varies from 32.81 to 34.65 per cent.; SO_4 from 5.95 to 6.82 per cent.; and Mg from 0.57 to 3.18 per cent. The main body of the lake supports no vegetation except blue-green algae, of which *Apanothece utahensis* is at present dominant. Three species of animals are listed by FLOWERS, of which one is *Artemia gracilis*. *Ruppia maritima* is reported as growing in Willard Spur, a part of Bear River Bay, where the strong brines of the main body of the lake are considerably diluted.

While magnesium in minute amounts is essential for all plants, to many it is toxic in very slightly increased amounts. Considerable variation is shown by different plants. EILERS (5), investigating the physiology of nutrition of the alga *Stichococcus bacillaris,* found that this was susceptible to relatively small amounts of magnesium salts. CARMIN (3) grew very young wheat seedlings in solutions of 0.0005 to 0.0150 M solutions of $MgSO_4$. When growth of the roots and tops was plotted against the concentration of $MgSO_4$, the curves showed lessening of the growth with greater concentration. The salt was more toxic to the roots than to the tops. TRELEASE and TRELEASE (36) detected injury due to magnesium toxicity in seedlings of wheat, oats, rye, and barley. The severity of injury increased as the Mg/Ca ratio increased from 1 to 50, showing the injury to be the result of disturbance of balance among the ions.

Very different results were obtained by ESPINO and PALISOC (6), who found that young rice plants grow well in a culture solution containing one part KH_2PO_4, one part $(NH_4)_2SO_4$, one part $Ca(NO_3)_2$, and thirty-two parts $MgSO_4$ with a total concentration of 0.00875 mols of salt per liter. $MgSO_4$ was necessary, apparently, as $Mg(NO_3)_2$ and $MgHPO_4$ produced plants with dry and chlorotic tips on the leaves.

WORONICHIN (39) reports a preliminary investigation of the relation of $MgSO_4$ deposit on the bottoms of lakes near Pjatigorsk to the plant growth in the lake. Three periods of development are described. In the first period, one of low and medium amounts of salt content, there is a growth of *Ruppia*. In the second period there is a decline in the *Ruppia* and vegetative growth of *Vaucheria dichotoma*. In the third period there is a rapid salting, decline of *Vaucheria* and the building of felt. Several new Myxophyceae are described: *Synechocystis minuscula, Oscillatoria*

tambi, and *Oscillatoria kützingiana* var. *crassa.* A green alga, *Carteria flos aquae,* is also listed.

Collection of material

Collecting trips were made to lake no. 1 at three different times. The first was in May of 1931, after several weeks of dry weather. The lake was reduced to a series of pools in which the water was greatly concentrated. The banks were brilliantly white, appearing to be practically pure salt deposits, although digging below the surface showed the mud underneath to be black in appearance and very ill-smelling. At the time of the first visit there was an abundant growth of seed-bearing *Ruppia maritima* and blue-green and green algae in the water. Great numbers of a small red crustacean, the "brine shrimp," *Artemia salina,* were swimming in the water. No plant collections were made, but several liters of water were taken and some of the mud was collected. When dried this was white and crystalline, being nearly identical in appearance with the salts obtained by evaporation of the water. It is in this mud, with its dry, white top layer, that the plants root. As the season advances, progressively deeper layers dry and become white, finally leaving the root system of *Salicornia* and other plants in what appears to be pure salt.

The second trip was on May 19, 1933. The season had been much rainier than that of two years earlier, and a fairly heavy rain had fallen the day before the trip was made. The pools of the lake were connected, the salt concentration in the water was much less than at the previous visit, and the banks, instead of being brilliant and white, were dull gray in appearance, giving evidence of their being composed of more than pure salt. *Ruppia maritima* was growing in even greater abundance than previously and was flowering, but there was little algal growth except in a small pool at one side, where the concentration was greater. *Artemia salina* was abundant in the lake, and a small red Cyclops was also present. Collections were made both of *Ruppia* and of the water in which it was growing.

The third collection was made July 22, 1933. Again the lake had evaporated to a series of pools, the banks around were white, and the water concentrated, although not so greatly as at the visit in 1931. *Ruppia* was growing somewhat less luxuriantly than in May and with a greater admixture of blue-green and green algae. The *Ruppia* had developed seeds about 1–2 mm. in diameter, black and shiny in appearance. Collections were made of the plant material and of the water.

Lake no. 2 was visited in May but no collections were made. The lake was continuous at that time, with grayish white banks. Occasional piles of salt on the bank were snowy in appearance. Except for a few algae, there was no sign of plant life in the waters of the lake.

The lake was visited again in July, 1933. The bed was an expanse of white salt with numerous round pools of saturated water, many of which were nearly covered with layers of salt which had crystallized out on top of the water. No phanerogams were found in the lake, but small numbers of a minute, unicellular, motile, yellowish green alga were seen, probably accounting for the yellowish green appearance of the pools from the hill above. Collections were made of the water and the alga, but the alga was not determined.

On the July trip to lakes no. 1 and no. 2 a stop was made at lake no. 3 and water was collected.

All three of the lakes are highly poisonous to the cattle which graze in the region and are tightly fenced to protect stock from access to them. Several carcasses lying within a few hundred feet of the lakes were testimony to the rapidity with which death followed when an animal did drink the water.

That the accumulation of salts is due to the lack of outlets usual to lakes is evidenced by the fact that some of the lakes lying between no. 2 and no. 3 were supporting an abundant growth of plants usual to lake waters and were left accessible to the stock in the region.

Material and methods

Data were obtained on the water and plant material from the three collections of May, 1931, May, 1933, and July, 1933. This included five samples of water, three from lake no. 1, one from lake no. 2, and one from lake no. 3. The plant collections included those of May, 1933, from lake no. 1 and those of July from lake no. 1. The specific gravity of the water was measured at 26° C. The pH was determined by means of a quinhydrone electrode. Concentration of dissolved substances was obtained by evaporation and drying at 100° C. The osmotic pressure was determined by measurement of the freezing point with a Beckmann thermometer. Ash analyses were made, using the methods of the Official Association of Agricultural Chemists, for Mg, Na, K, Ca, Fe and Al, SO_4, Cl, PO_4, and NO_3. No analyses were made for sulphide or sulphite, although traces of sulphide were undoubtedly present.

The plant material was washed thoroughly with tap water, dried at 63° C., and the moisture content determined.

The soluble portion of the plant was extracted by boiling and expressing the sap by pressure. The sample was weighed, distilled water was added and boiled, and the sap was pressed out. Then the whole extract was evaporated to the volume of the original sap as determined by weight of the original sample and weight of the insoluble material. Measurements were then made of the specific gravity, pH, and osmotic pressure of the sap.

Determinations were made for soluble sugars in the sap by the Shaffer-Hartmann method and for protein N by the Kjeldahl method. Ash analyses were made on both the sap and the insoluble residue for Mg, Fe and Al, Na, K, Ca, Cl, SO_4, PO_4, and NO_3.

As it was thought possible that arsenic might be found in some of the lakes, qualitative analyses were made for it using Marsh's test. No traces were found in any of the five samples analyzed.

In addition to these data, some observations were made on the growth of *Ruppia* and of *Artemia* from the May, 1933, collections in different culture solutions. The solutions were made up as follows: lake water, from the May, 1933, collection from lake no. 1; equal parts of lake water and distilled water; two-thirds lake water and one-third 2 per cent. $MgCl_2$ solution; one-third lake water and two-thirds 2 per cent. $MgCl_2$ solution; Knop's solution; and Knop's solution with additional $MgCl_2$ to bring the osmotic pressure up to that of the lake water. About a quart of each solution was placed in a 2-quart glass jar and a few plants of *Ruppia* were placed in each. Records were made of the vigor of the plants as well as this could be determined by observation.

Three specimens of *Artemia*, a small amount of algal material from lake no. 1, and some culture solution were placed in each of a group of small beakers, and observations were made of the behavior of the crustacean. The culture solutions were as follows: lake water, from the May collection of 1933; one-half lake water and one-half 4 per cent. $MgCl_2$ solution; 2 per cent. $MgCl_2$ solution; 4 per cent. $MgCl_2$ solution; 12 per cent. $MgCl_2$ solution; distilled water; and 22 per cent. solution of salts obtained from evaporation of the water collected in May, 1931.

Data

Analyses of the water samples for NH_3 showed traces in the water. Data accumulated in measurements and analyses of the samples of water and of plant material are listed in tables I to IX.

Figures obtained for the moisture content of the plants were: May collection, 92.2 per cent.; July collection, 90.8 per cent.

The cultures of *Ruppia* showed varying results. None of the plants remained alive after 45 days. Death at that time was probably due to their being placed in rather narrow-mouthed jars with no special care taken to provide for aeration. Those in Knop's solution were the first to succumb, being dead and decomposing within 15 days. Those in Knop's solution and $MgCl_2$ with an osmotic pressure equal to that of the lake water survived only a few days longer than the plants in Knop's solution alone. The plants in two-thirds lake water and one-third 2 per cent. $MgCl_2$ behaved much the same as those in one-third lake water and two-thirds 2 per cent. $MgCl_2$

TABLE I

WATER COLLECTED FROM LAKE NO. 1, MAY, 1931. APPROXIMATE CONCENTRATION, 26%; SPECIFIC GRAVITY AT 23° C., 1.21; OSMOTIC PRESSURE, 58.0387 ATM.; pH, 7.4

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	36.6145	1.5055	3.0110	40.1	30250
Na	8.60	0.3739	0.3739	9.96	7100
K	1.6651	0.0426	0.0426	1.13	1380
Ca	0.0912	0.0023	.0046	0.06	75
Fe and Al	Trace				
Total	46.9708	1.9243	3.4321	51.25	38805
SO_4	169.7694	1.7684	3.5368	47.16	140305
Cl	1.935	0.0546	0.0546	1.45	1600
NO_3	0.1429	0.0023	0.0023	0.06	120
PO_4	0.0131	0.0001	0.0003	.	10
Total	171.8604	1.8254	3.5940	48.67	142035

Total molar concentration, 3.7497 mols per liter.

TABLE II

WATER COLLECTED FROM LAKE NO. 1, MAY, 1933. APPROXIMATE CONCENTRATION, 1.6%; SPECIFIC GRAVITY AT 26° C., 1.012; OSMOTIC PRESSURE, 3.797 ATM.; pH, 8.4

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	2.1207	0.0872	0.1744	38.98	2100
Na	0.6161	0.0268	0.0268	11.98	610
K	0.0981	0.0025	0.0025	1.12	97
Ca	0.0084	0.0002	0.0004	0.08	8
Fe and Al	Trace				
Total	2.8433	0.1167	0.2041	52.16	2815
SO_4	9.6902	0.1009	0.2018	45.10	9575
Cl	0.2070	0.0058	0.0058	2.59	200
NO_3	0.0142	0.0002	0.0002	0.08	15
PO_4	0.0007	. .	.	. .	
Total	9.9121	0.1069	0.2078	47.77	9790

Total molar concentration, 0.2236 mols per liter.

TABLE III

WATER COLLECTED FROM LAKE NO. 1, JULY, 1933. APPROXIMATE CONCENTRATION, 6%; SPECIFIC GRAVITY AT 26° C., 1.045; OSMOTIC PRESSURE, 12.28 ATM.; pH, ABOVE 8.5

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	10.3757	0.4266	0.8532	39.89	9930
Na	2.3050	0.1002	0.1002	9.37	2200
K	0.4557	0.0116	0.0116	1.08	440
Ca	0.0027	0.0001	0.0002	0.01	3
Fe and Al	Trace				
Total	13.1391	0.5385	0.9652	50.35	12573
SO_4	49.4723	0.5153	1.0306	48.18	47340
Cl	0.544	0.0153	0.0153	1.43	520
NO_4	0.0196	0.0003	0.0003	0.03	20
PO_4	0.0026		. ..	.	2
Total	50.0385	0.5309	1.0462	49.64	47882

Total molar concentration, 1.0694 mols per liter.

TABLE IV

WATER COLLECTED FROM LAKE NO. 2, JULY, 1933. APPROXIMATE CONCENTRATION, 48%; SPECIFIC GRAVITY AT 26° C., 1.380; OSMOTIC PRESSURE,* 125.80 ATM.; pH, 7.28

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	46.565	1.9146	3.8292	24.45	33740
Na	51.5239	2.2402	2.2402	28.61	37330
K	11.3754	0.2909	0.2909	3.71	8240
Ca	0.0009	.	. .		....
Fe and Al					
Total	109.4652	4.4457	6.3603	56.77	79310
SO_4	314.15	3.2724	6.5448	41.79	227644
Cl	3.42	0.1123	0.1123	1.43	2480
NO_3	0.0047	0.0001	0.0001		3
PO_4	0.0247	0.0003	0.0009	...	18
Total	317.5994	3.3851	6.6581	43.22	230145

Total molar concentration, 7.8308 mols per liter.

* To measure the freezing point depression it was found necessary to dilute the water to one-fourth the original concentration. The pressure given is calculated on that basis and is somewhat high.

TABLE V

WATER COLLECTED FROM LAKE NO. 3, JULY, 1933. APPROXIMATE CONCENTRATION, 2.4%; SPECIFIC GRAVITY AT 26° C., 1.020; OSMOTIC PRESSURE, 6.506 ATM.; pH, ABOVE 8.5

	Ash analysis				
	Grams per liter	Mols per liter	Equivalents per liter	Total mol conc.	P.P.M.
	gm.			%	
Mg	2.8152	0.1158	0.2316	32.53	2760
Na	1.312	0.0570	0.0570	16.01	1290
K	0.7704	0.0197	0.0197	5.53	755
Ca	0.0124	0.0031	0.0062	0.87	12
Fe and Al	Trace	..			.
Total	4.9100	0.1956	0.3145	54.94	4817
SO_4	14.6053	0.1503	0.3006	42.22	14320
Cl	0.3472	0.0098	0.0098	2.75	340
NO_3	0.0106	0.0002	0.0002	0.05	10
PO_4	0.0128	0.0001	0.0003	0.03	12
Total	14.9759	0.1604	0.3109	45.05	14682

Total molar concentration, 0.3560 mols per liter.

TABLE VI

PLANT SAP FROM COLLECTION OF MAY, 1933, LAKE NO. 1. SPECIFIC GRAVITY AT 26° C., 1.020; SOLUBLE SUGARS, 1.109 MG. GLUCOSE PER CC.; OSMOTIC PRESSURE, 6.637 ATM.; pH, 6.12; SOLUBLE PROTEIN NITROGEN, 0.27%

	Ash analysis				
	Grams per liter	Mols per liter	Equivalents per liter	Total mol conc.	P.P.M.
	gm.			%	
Mg	1.876	0.0771	0.1542	31.40	1840
Na	0.4746	0.0206	0.0206	8.39	465
K	0.2800	0.0072	0.0072	2.93	275
Ca	0.3494	0.0087	0.0174	3.54	340
Fe and Al	Trace				
Total	2.9800	0.1136	0.1994	46.26	2920
SO_4	6.693	0.0697	0.1394	28.39	6560
Cl	1.595	0.045	0.045	18.33	1560
NO_3	0.9876	0.016	0.016	6.51	970
PO_4	0.1135	0.0012	0.0036	0.48	110
Total	9.3891	0.1319	0.2040	53.71	9200

Total molar concentration, 0.2455 mols per liter.

TABLE VII

INSOLUBLE PLANT MATERIAL FROM COLLECTION OF MAY, 1933, LAKE NO. 1

	Ash analysis	
	Grams per 100 grams dried insoluble residue	Insoluble material
	gm.	%
Mg	1.8404	1.84
Na	0.3600	0.36
K	0.2124	0.21
Ca	1.1369	1.14
Fe and Al	Trace	
Total	3.5497	3.55
SO_4	2.36	2.36
Cl	0.6588	0.66
NO_3	0.7088	0.71
PO_4	0.0011	..
Total	3.7287	3.73

TABLE VIII

PLANT SAP FROM COLLECTION OF JULY, 1933, LAKE NO. 1. SPECIFIC GRAVITY AT 26° C., 1.025; SOLUBLE SUGARS, 1.06 MG. GLUCOSE PER CC.; OSMOTIC PRESSURE, 9.755 ATM.; SOLUBLE PROTEIN NITROGEN, 0.51%

	Ash analysis				
	Grams per liter	Mols per liter	Equivalents per liter	Total mol conc.	P.P.M.
	gm.			%	
Mg	2.936	0.1207	0.2414	27.48	2865
Na	1.2668	0.0551	0.0551	12.54	1235
K	0.8817	0.0225	0.0225	5.12	860
Ca	0.4237	0.0106	0.0212	2.41	415
Fe and Al	None				
Total	6.5082	0.2089	0.3402	47.55	5375
SO_4	15.120	0.1575	0.3150	35.86	14750
Cl	2.0409	0.0576	0.0576	13.11	1990
NO_3	0.5128	0.0054	0.0054	1.22	500
PO_4	0.9356	0.0098	0.0294	2.23	910
Total	18.6093	0.2303	0.4074	52.42	18150

Total molar concentration, 0.4392 mols per liter.

TABLE IX

INSOLUBLE PLANT MATERIAL FROM COLLECTION OF JULY, 1933, LAKE NO. 1

	Ash analysis	
	Grams per 100 grams dried insoluble residue	Insoluble material
	gm.	%
Mg	0.619	0.62
Na	0.8108	0.81
K	0.4783	0.48
Ca	0.6151	0.62
Fe and Al	. .	
Total	2.5232	2.53
SO_4	5.845	5.84
Cl	0.1093	0.11
NO_3	0.3544	0.35
PO_4	0.6128	0.62
Total	6.9215	6.92

solution, showing a slight browning and a lessening in oxygen given off within two days. Death was gradual, however. The brown color increased slowly and at the end of 40 days some of the plants were still green. Likewise, little difference was shown between the plants grown in normal lake water and those grown in half lake water and half distilled water. These groups survived the best, being apparently healthy at the end of 16 days. By the end of 40 days they were showing some brown color, although somewhat less than the others, and shortly after they were also dead. It is interesting to note that a heavy growth of algae took place in the jars upon death of the *Ruppia*. No attempt was made to identify these, but the jars which contained Knop's solution and no lake water showed what appeared to be primarily a Chlorophycean growth, while those with the lake water appeared to have a Myxophycean growth.

Artemia was much quicker in responding to differences of environment. Those allowed to remain in lake water were not affected, being alive and showing no evidence of sluggishness at the end of 15 days, when the observations were discontinued. Those in half lake water and half 4 per cent. $MgCl_2$ solution were less affected than most of the others. At the end of 24 hours they were somewhat sluggish but recovered from this and survived for some days. At the end of six days one had died, and in four days more all were dead. The specimens placed in 22 per cent. salt from lake no. 1 survived for four days, when two died. The remaining one lived two days longer. Distilled water was the most unfavorable medium. Two of

the specimens were dead within five hours; the remaining one was sluggish and died within another three hours. Those in 2 per cent. $MgCl_2$ solution survived slightly better but were slow in movement at the end of eight hours and dead within 24 hours. Two of the *Artemia* in 4 per cent. $MgCl_2$ solution died within five hours; the other, although not active, was alive at the end of 24 hours but died a few hours later. Those in 12 per cent. $MgCl_2$ did not succumb until nearly 48 hours had passed. Those of the organisms which had died in solutions containing no $MgCl_2$ lost the red color but remained colorless after death. Those in the solutions containing $MgCl_2$ not only lost the red pigment upon dying but turned black. The black color appeared first along the mid part of the dorsal side and gradually spread out through the rest of the crustacean.

Analysis of data and conclusions

Comparison of the samples collected from lake no. 1 (tables I, II, III) shows variation in concentration from 1.6 to 26 per cent. at different seasons. *Ruppia maritima* is able to grow well under these concentrations, although the most luxuriant vegetative growth was at the lower concentration. The fact that the *Ruppia* had already produced seeds in May, 1931, when the concentration was very high, while the plant was flowering in May, 1933, at a concentration of slightly more than 1.5 per cent., indicates that fruiting is more dependent upon concentration than upon the season. The increase in concentration from 1.5 per cent. in May to 6 per cent. in July was accompanied by the development of the seeds to maturity. The algal growth, particularly of blue-green algae, was more abundant in the solutions of greater concentration.

Comparison of the salt contents of the three collections shows only small variations in the relative proportions of ions present. The Mg content is almost constant while that of the SO_4, although somewhat higher than the Mg, is only slightly more variable. These total between 84 and 88 per cent. of the total salt analyzed. While the K content remains practically the same for the two May collections and drops slightly for the July sample, the Na and Cl are both slightly greater for the dilute waters of the May, 1933, sample. Ca, at all times scant in amount, drops in the July collection. NO_3 behaves much as Ca; and Fe, Al, and PO_4 are at all times negligible in amount.

Table IV shows the analysis of lake no. 2. Here the concentration has reached the saturation point, so that, even on the hot July day when the collection was made, crystallization of salts had partially covered the water with a layer of salt. In this lake Ca, Fe, Al, NO_3, and PO_4 are practically lacking. The amount of Cl is about the same as that in tables I and III. Although the amount of SO_4 is somewhat less than that in lake no. 1, the

greatest difference lies in the amounts of Mg, Na, and K. The Mg content is about three-fifths that of lake no. 1 while the Na is almost three times as great. And the K is slightly more than three times as great as in lake no. 1. As lake no. 2 showed no organic life except the unidentified alga, the failure of plant life to grow there, when it is abundant in lake no. 1, might be due to the extreme concentration of total salt, to the lack of the essential Ca, PO_4, NO_3, and Fe, or to the greater proportion of K and Na in the solution.

The analyses of the third lake are given in table V. The concentration (2.4 per cent.) is less than in either of the other lakes in July, although greater than lake no. 1 in May, 1933. There are traces of Fe and Al. NO_3, PO_4, and Ca especially are more abundant than in lake no. 1. SO_4 is present in nearly the same amount (42.22 per cent.) as in lake no. 2. The proportions of Mg, Na, and K are again different. There is about four-fifths as much Mg as in lake no. 1 in July, somewhat less than twice as much Na, and about five times as much K. With comparisons of the actual amounts in the water from lake no. 1 in May, 1933, and lake no. 3 (which are not greatly different in concentration), it is seen that slightly more Mg and much more Na and K are present in lake no. 3. The amounts are nevertheless much smaller in either of these samples than in the water of lake no. 1 in May, 1931, or July, 1933, when plants were growing abundantly. Comparison of the three lakes suggests that the toxicity of lakes 2 and 3 to plant life lies neither in the high concentration nor in the lack of Ca, NO_3, and PO_4, but rather in the relatively high proportion of Na and K in the total salt content.

This conclusion is supported by the distribution of plant life in Great Salt Lake, as reported by FLOWERS (8). The plant life of the lake is entirely algal, although the concentration reaches no greater value than that of the magnesium sulphate lake no. 1 described here. The chief constituent, however, is NaCl rather than $MgSO_4$. The growth of *Ruppia* in less concentrated bays of Great Salt Lake shows that concentration of NaCl is evidently the limiting factor.

Tables VI and VII show the results of analyses on the soluble sap and the insoluble residue of the plant material collected in May, 1933. Tables VIII and IX show the results of analyses of the July collection. These show a high content of both Mg and SO_4. This may be due in part to failure to remove all of the external $MgSO_4$ in washing preparatory to extracting the sap, but the content is high in the July collection as well as the May. As the former was washed even more thoroughly than the latter, the amount of salt due to external $MgSO_4$ is probably not great.

Comparison of the sap of *Ruppia* from the May collection with the July collection shows a slight decrease of soluble sugars and an increase of soluble

protein nitrogen. The total molar concentration of the salts for which analyses were made has increased from 0.2455 to 0.4392 mols per liter. Tables VI and VIII show the actual amount of salt to have increased during the period from May to July in all cases except that of NO_3. This loss of NO_3 is probably due to the increase in protein nitrogen. The relative molar concentrations have changed considerably. There is a decrease in the Mg and Ca and an increase of Na and K. Likewise the SO_4 and PO_4 have increased, the latter greatly, while the Cl and NO_3 have decreased.

Selective absorption of ions by the plant is shown by the concentrations of different ions in the plant sap and the water in which it is growing. Tables II and VI give these values for the May samples. Mg and SO_4 are high in both, showing the plant able to tolerate these internally, but the amount is greater in the water than in the sap. The water contains 2.1207 gm. of Mg and 9.6902 gm. of SO_4 per liter while the sap contains 1.876 gm. of Mg and 6.693 gm. of SO_4 per liter. The water contains 0.6161 gm. of Na per liter while the sap contains 0.4746 gm. per liter. The remainder of the ions, especially Ca, NO_3, and PO_4, are more abundant in the plant sap than in the water. While there are 0.0981 gm. per liter of K and 0.2070 gm. per liter of Cl in the water, the sap contains 0.2800 gm. of K and 1.595 gm. of Cl. The water contains 0.0084 gm. and the sap contains 0.3494 gm. of Ca per liter; the water contains 0.0142 gm. and the sap 0.9876 gm. of NO_3 per liter; the water contains 0.0007 gm. and the sap 0.1135 gm. of PO_4 per liter.

Similar results are shown by comparison of the sap of the July collection and the water (tables III, VIII), although the concentrations are greater in both sap and water than in the May material. The water contains 10.3757 gm. of Mg. 49.4723 gm. of SO_4, and 2.3050 gm. of Na per liter while the plant contains 2.936 gm. of Mg, 15.120 gm. of SO_4, and 1.2668 gm. of Na per liter. As in the May material, the other ions are more abundant in the plant than in the water. The water contains 0.4557 gm. of K, 0.0027 gm. of Ca, 0.544 gm. of Cl, 0.0196 gm. of NO_3, and 0.0026 gm. of PO_4 while the plant sap contains 0.8817 gm. of K, 0.4237 gm. of Ca, 2.0409 gm. of Cl, 0.5128 gm. of NO_3, and 0.9356 gm. of PO_4. There has been considerable change in the ratios of material in the plant to material in the water from those shown in the May material. While the ratios of K, of Cl, and of NO_3 in the sap to that in the water have decreased from May to July, the ratios of Ca and of PO_4 in the sap to that in the water have increased greatly.

The contents of both plant sap and water may be compared with those of plants growing in sea water and in pond water. OSTERHOUT (**26**) reports the concentration of *Valonia* and of the sea water in which it grows. His results, converted from parts per thousand to parts per million for purposes of comparison, are given in table X.

TABLE X

	Conc. in sea water	Conc. in cell sap
	p.p.m.	*p.p.m.*
Mg	1310	Trace
Na	10900	2100
K	500	20100
Ca	450	700
SO_4	3300	5
Cl	19600	21200

With regard to the water, the difference lies chiefly in high NaCl in the sea water and high $MgSO_4$ in the lakes. The plant sap analyses are interesting. *Valonia* excludes Mg and SO_4 almost entirely and allows little Na to enter, although the concentration is high in the water. Cl and Ca are absorbed in somewhat greater concentration, and K is many times more concentrated than in the water.

In *Ruppia* the sap shows many times as much PO_4, NO_3, Ca, and K as the water. Cl is likewise selectively absorbed by the plant and Na is excluded to some extent. Unlike *Valonia, Ruppia* is able to tolerate Mg and SO_4 in large quantities, allowing a greater amount of these than any of the other ions to enter. Hoagland and Davis (9) made analyses of *Nitella clavata* which was growing in pond water (table XI).

TABLE XI

	Weight	
	Water	Cell sap
	p.p.m.	*p.p.m.*
Mg	41	430
Na	5	230
K	Trace	2120
Ca	31	410
SO_4	31	800
Cl	32	3220
NO_3	34	0
PO_4	0.4	350

In this case the amounts of Mg, SO_4, and Cl are not greatly different from one another and approximate the amounts of Ca and NO_3 in the water. Na is small in amount. The greatest difference from the water of lake no. 1 is in the much smaller concentration of total salts. While some Mg and SO_4 have been taken up by *Nitella,* the amount is much less than that tolerated by *Ruppia,* while the amounts of K and Cl are much greater.

Determinations of the pH of the five samples of lake water show all of them to be alkaline, three of them being 8.4 or above. Apparently the pH of the lakes has little influence on plant growth, as one of the lower readings (7.4) was obtained from the May, 1931, collection from lake no. 1 where plants were abundant, and the other (7.28) from the highly concentrated water of lake no. 2 where very little life was found.

Osmotic concentration determinations show the water samples to vary from 3.797 atmospheres in lake no. 1 in May, 1933, to nearly 125 atmospheres in lake no. 2 in July, 1933. In July the osmotic pressure of lake no. 1 was 12.28 atmospheres. The osmotic concentration of lake no. 1 in May of 1931, the most concentrated sample in which *Ruppia* was growing, was 58.0387 atmospheres.

The plant sap determined in May, 1933, had an osmotic concentration equal to 6.637 atmospheres, or 2.84 atmospheres more than the water surrounding it. By July the concentration of the plant sap had increased to 9.755 atmospheres. In the meantime, however, the concentration of the water had increased until the equivalent osmotic pressure was 12.28 atmospheres, or 2.525 atmospheres above that of the plant. It was not possible to determine, from the collections made, the stage of development of plants of *Ruppia* when the osmotic pressures shifted to make the external greater than the internal pressure.

The responses of *Ruppia* and *Artemia* to different culture conditions indicate that SO_4 is necessary when Mg is present, as the substitution of Cl for SO_4 resulted in rather quick death for *Artemia* and somewhat slower death for *Ruppia.* Distilled water was quickly fatal for *Artemia;* and Knop's solution, a satisfactory culture solution for most plants, caused or allowed *Ruppia* to die within a rather short period. Apparently these organisms are growing in unusual conditions, not only because they survive where other plants and animals cannot but also because they themselves require unusual conditions for growth. It is of interest, in connection with the study of *Artemia* in different culture solutions, to review the work of BOONE and BAAS-BECKING (**1**). They report KCl as being very toxic to *Artemia salina* although the adult *Artemia* survives in solutions of $MgCl_2$, $CaCl_2$, HCl, and NaCl. Only NaCl and NaBr solutions are found to cause normal ecdysis, even the chlorides of Ca and Mg being toxic after a few days.

The range of concentrations reported as being suitable for life in *Artemia* is 0 to 3.5 mols. BOONE and BAAS-BECKING (**1**) state that increasing Mg content of the brine is probably a factor in the disappearance of *Artemia.* These conclusions agree with those arrived at by growing *Artemia* in so far as the eventual toxicity of $MgCl_2$ is concerned. However, in view of the fact that *Artemia* was growing in the water collected in May, 1931,

which had a concentration of not less than 3.75 mols, of which 40 per cent. was Mg, it seems necessary to extend the range of suitable concentrations somewhat and to look for some factor other than the increase of Mg as cause for the disappearance of *Artemia.*

The halophyte *Ruppia maritima* presents an example of a plant growing directly in water but under conditions of great "physiological dryness," as the concentration of salts in the water produces osmotic pressure of 58 atmospheres or more without causing the death of the plant. *Ruppia* is able to increase the internal osmotic pressure, by utilization of salts from the water surrounding it, to greater values than the external pressure. At some stage between the external pressures of 3.797 and 12.28 atmospheres, the internal osmotic pressure of *Ruppia* fails to increase as rapidly as the external pressure, resulting in greater external than internal pressure. Further study might show this shift of balance in external and internal pressures to coincide with maturation of the seeds and cessation of vegetative growth in *Ruppia.*

Ruppia is unusual in being able to tolerate magnesium sulphate in high concentrations. The percentage of Mg and SO_4 in the water surrounding *Ruppia* was 84 to 88 per cent. of the total salt content. The plant does not exclude either Mg or SO_4. On the contrary, both are found in the plant sap in greater proportionate amounts than any other ions for which analyses were made, although not in so great concentration, compared with other ions, as in the water.

Ruppia maritima, although able to develop successfully in lakes of high magnesium sulphate content, does not tolerate high concentration of NaCl. It does not grow in Great Salt Lake, where the concentration is comparable with that of lake no. 1 but where the chief constituent is NaCl. *Ruppia* is likewise not found in either of two lakes near lake no. 1, one of which shows very high concentration and the other low concentration but both of which have relatively more Na and K. Na is evidently much more toxic to *Ruppia* than is Mg. Cultures of *Ruppia* in $MgCl_2$ solutions indicate the necessity of SO_4 with Mg, suggesting an antagonism of SO_4 and Mg ions.

Summary

1. Collections were made from a lake supporting *Ruppia* in May, 1931, May, 1933, and July, 1933.

2. At the time of the first collection, the concentration of the lake water was about 26 per cent., the pH was 7.4, the specific gravity was 1.21 at 23° C., and the osmotic equivalent was 58.0387 atmospheres. *Ruppia* was abundant, there was a heavy growth of blue-green and green algae, and the crustacean *Artemia salina* was abundant. The banks of the lake were dry, crystalline, and white on top with black mud underlying. Collections were

made of the water and of the mud. Upon drying, this mud appeared white and crystalline like the layer above.

3. At the time of the second collection, May, 1933, after a rainy season, the concentration of the lake was about 1.6 per cent., the pH was 8.4, the specific gravity was 1.012 at 26° C., and the osmotic pressure was 3.797 atmospheres. *Ruppia* showed a heavy vegetative growth and was flowering. *Artemia* was abundant, but fewer algae were found than at other visits. The banks were gray and dull in appearance. Collections were made of water, of *Ruppia,* and of *Artemia.*

4. At the time of the third collection, July, 1933, after dry weather, the concentration was about 6 per cent., the pH was above 8.5, the specific gravity was 1.045 at 26° C., and the osmotic pressure was 12.28 atmospheres. Algae, *Artemia,* and seed-bearing *Ruppia* were abundant. The banks were white and crystalline in appearance. Collections were made of *Ruppia* and of water.

5. Collections of water were made at the other two lakes in July, 1933. The concentration of one lake was about 48 per cent., the pH was 7.28, the specific gravity was 1.380 at 26° C., and the osmotic pressure was about 125 atmospheres. The lake had been reduced by evaporation to many small round pools with salt crystallized on top, and the lake bed was white and crystalline with black mud under the surface layer. The concentration of the other lake was about 2.4 per cent., the pH was above 8.5, the specific gravity was 1.020 at 26° C., and the osmotic pressure was 6.506 atmospheres.

6. Sap from plants of the May collection showed a specific gravity of 1.020 at 26° C. and an osmotic pressure of 6.637 atmospheres; from the July collection, a specific gravity of 1.025 at 26° C. and an osmotic pressure of 9.755 atmospheres.

7. Quantitative analyses were made for Mg, Na, K, Ca, Fe and Al, SO_4, Cl, PO_4, and NO_3 in the sap and insoluble material of the plant collections and in each of the five water samples. Analyses were made of the water samples for NH_3, showing traces present, and for As, showing no trace of any present.

8. The analyses show 40 per cent. of the total molar concentration of the salt in the lake supporting *Ruppia* to be due to Mg; 45 to 48 per cent. to be due to SO_4; and 9 to 11 per cent. to be due to Na.

9. The pH is apparently not a factor in plant distribution in these lakes.

10. *Ruppia* is able to tolerate Mg and SO_4 in high concentrations internally as well as externally, evidently using these in developing its own osmotic pressure.

11. The limiting factor in the development of *Ruppia* in lakes not supporting it is evidently a greater proportion of Na_2SO_4.

12. Cultures of *Artemia* and *Ruppia* indicate that $MgCl_2$ cannot replace $MgSO_4$.

13. Time of flowering and seed development of *Ruppia* are evidently influenced by the concentration of the lake in which it grows.

14. *Ruppia* was growing in external osmotic pressures of 3.797 atmospheres with an internal pressure of 6.637 atmospheres, and 12.28 atmospheres with an internal pressure of 9.755 atmospheres.

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PHYSIOLOGICAL RÔLE OF ASPARAGINE AND RELATED SUBSTANCES IN NITROGEN METABOLISM OF PLANTS[1]

A. E. Murneek

Historical and theoretical retrospect

Asparagine, the amide of amino succinic acid, $COOH \cdot CHNH_2 \cdot CH_2 \cdot CONH_2$, seems to have been found in asparagus over one hundred years ago (**50**) and so named soon thereafter. Mothes (**14**) credits Hartig (1858) as having extracted this substance from seedlings and isolated it for the first time in crystalline form. He thought that asparagine was a translocation product of nitrogen in young plant tissues. Boussingault (**2**), however, assigned to it a different function by expressing the belief that there is an analogy between asparagine and urea in nitrogen metabolism, both being amides and end products of protein degradation. But urea is excreted from the animal organism, while asparagine is not eliminated from plants. In presence of light, it is reutilized for the synthesis of proteins. These hypotheses of Hartig and Boussingault are still both supported and contended.

After extensive microchemical studies on the presence and absence of asparagine in all parts of a large number of plants, Pfeffer (1872) attempted to unite the two opposing views by concluding that: (1) Protein degradation in plants, because of formation of asparagine, differs from that in animals and from results obtained *in vitro*. (2) Asparagine is the primary, not the end product of protein breakdown and should be considered also a storage form of nitrogen. (3) Judging from its presence and solubility, it is the main translocation product of nitrogenous substances. It moves to growing regions, where it combines with carbohydrates (glucose) to form proteins. (Of interest in this respect is the fact that Chibnall (**4**) still seems to agree with Pfeffer that asparagine is the chief form in which nitrogen is translocated in plants).

Schulze (**36, 37, 38, 39, 42, 43, 44, 45**) for 30 years studied many phases of nitrogen metabolism in plants, with particular reference to asparagine, by physiological and chemical means. While he was unsuccessful in demonstrating an *in vivo* and *in vitro* parallelism of protein hydrolysis and asparagine accumulation, he did show definitely that under normal conditions asparagine is produced from proteins and not from inorganic nitrogen sources. But through further studies, largely with seedlings of legumes,

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SCHULZE and later his coworkers (primarily PRIANISCHNIKOV and associates) supplied evidence in support of the hypothesis that asparagine may be formed readily from ammonia and organic acids.

SCHULZE found that during early stages of germination and growth of seedlings, cotyledons contain only primary products of protein hydrolysis, the amino acids (leucine, tyrosine, etc.). During later development of the plant, amino acids disappear from the cotyledons and asparagine accumulates in large amounts in the shoots. Therefore asparagine does not seem to be produced directly from proteins,[2] but results from breaking down of the amino acids.

Through his own extensive investigations and those of many coworkers, PRIANISCHNIKOV (**20–31**) seems to have established the analogy between asparagine and urea in nitrogen metabolism. Moreover, he has demonstrated that asparagine may be synthesized directly in seedlings from substances within the plant and an external supply of ammonia or carbohydrates or both.

As a result of the preceding and many other studies, the following theoretical considerations seem to have become popular (**27, 6, 14,**):

> When, in comparison with carbohydrates, there is an excess of ammonia, or proteins are not required by the plant because of shortage of carbohydrates, then through hydrolysis of proteins by means of proteolytic enzymes amino acids are formed. Some of these (especially mono-amino acids) are oxidized and amino (NH_2) groups are released. Asparagine, which contains two NH_2 groups, is formed from two molecules of amino acids. One of these is oxidized to aspartic acid, the other much further with splitting off of ammonia. A union of aspartic acid with ammonia forms ammonium aspartate, from which, through dehydration, asparagine is produced in the same manner as in the animal organism urea is formed from carbamate of ammonia. When a plant is supplied with abundance of ammonia, asparagine may be formed from one molecule of an amino acid or even from an organic acid (malic, succinic, etc.). If there is need for nitrogen, asparagine will be broken down, NH_3 released, and the rest of the molecule may be oxidized to CO_2. Asparagine, therefore, has a dual function: removal of the injurious NH_3, and storage of N.

In some plants, or under some conditions in most plants, not only asparagine but *glutamine*, the amide of amino-glutaric acid (**41**), *argi-*

[2] There is the possibility, of course, that asparagine may exist as such in the protein molecule, as is suggested by SCHULZE, OSBORN, and BUTKEWITSCH. In acid hydrolysis of proteins the amide group is saponified, giving aspartic or glutamic acids; but when proteins are acted upon by proteolytic enzymes, the amides appear unaltered. In many instances, however, the quantity obtained from seedlings is entirely too large to be accounted for on this basis.

nine, guanidine-amino-valerianic acid (**39, 40**), *allantoin*[8] or allantoic acid (**1**), *urea,* $CO(NH_2)_2$(10), and possibly other substances may serve as receptors of NH_3. Organic acids (malic, succinic, oxalic) likewise may take care of ammonia by forming ammonium salts, which seem to be a characteristic feature of very acid plants.

When plants have an ample supply of carbohydrates (sugars) the process is reversed. Ammonia is released from any of the receptors and is used in the synthesis of amino acids, proteins, and other complex nitrogenous products.

Experimental evidence

No attempt, of course, is made here to present all the important evidence bearing on the subject under discussion. Only some of the typical results of investigations on certain parts of the major phases of the problem will be recounted. Those desiring to secure more complete information will naturally turn to the extensive literature in this field.

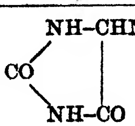
1. Nitrogen metabolism of seedlings

Changes in nitrogenous substances in the germinating seeds and seedlings have been thoroughly investigated by SCHULZE, PRIANISCHNIKOV, and others. This has involved a study of the breakdown of proteins in the cotyledons and regeneration of nitrogen compounds in the developing stem and leaves. A conspicuous feature, often emphasized in these studies, is the abundance of asparagine in the seedlings of legumes, especially when grown in darkness. It is evident from SCHULZE's and other work (**4**) that with increased development of the plant there is a decrease in protein concentration and a concomitant increase in asparagine and mono-amino acids (table I). But since the amino acid fraction decreases when the seedlings

TABLE I

NITROGEN METABOLISM IN LEGUME SEEDLINGS (SCHULZE)

AGE OF SEEDLINGS IN DAYS	PROTEIN	ASPARAGINE	OTHER N (MOSTLY MONO-AMINO ACIDS)
	%	%	%
6	5.49	1.16	1.72
12	1.71	4.02	2.39
24	1.78	5.09	1.40

[8] Allantoin, , a diuride of glyoxalic acid.

are of considerable age, while at the same time there is a proportional increase in asparagine and no further decrease in proteins, it appears that asparagine may have come from the amino acid fraction. These plants, of course, had no external supply of nitrogen.

Similar results have been obtained not only with several legumes but also with other seedlings by many workers. Asparagine accumulates likewise in onion bulbs, asparagus roots, and similar plant structures when they are sprouted in darkness (**54, 18, 32**).

The converse of this process takes place when, for instance, seedlings are grown in light and proteins are synthesized in presence of sufficient amount of carbohydrates, or in case of development of bulbs, whose sugar content is usually high (**54, 56**). In general, the higher the amount of soluble carbohydrates in the seeds, bulbs, or roots, the less protein will be broken down owing to respiration, when growth is resumed, and there will be relatively little fluctuation in asparagine concentration.

Most oil-containing seeds produce upon germination glutamine (**41**) instead of asparagine, but the general nitrogen transformations seem to be the same. In some plant seedlings other substances than acid amides appear to play a similar rôle, as will be noted further on.

2. Nitrogen metabolism in leaves

Several investigators have demonstrated that asparagine and its homologue, glutamine, perform the same function in nitrogen metabolism of leaves as in developing seedlings. In young rapidly growing leaves, both amides may be used in synthesis of proteins (**44**). In fully developed leaves proteins are formed during the day and may be broken down and removed in soluble forms of N to other parts of the plant. Chibnall (**4, 5**), for example, has demonstrated that asparagine disappears from the leaves of the bean plant at night and that it comes largely from hydrolysis of proteins. In fruit-bearing plants nitrogen may be translocated in this form to the fruit or seed pods whence eventually it will go into the seeds (**44, 45, 53**).

Alterations in nitrogen content of starved or senescent leaves supply additional evidence of the rôle of acid amides in plant metabolism. The results secured by Miyachi are presented here (table II). They show that when leaves are starved there is a rapid breakdown of proteins and a striking increase in asparagine and other amides. Similarly, Vickery (**52**) has found that during the curing of tobacco leaves, with increasing loss of amino-N there is a more or less corresponding gain in amide-N (table III). He thinks that such change may be due to the oxidation of amino acids, the formation of ammonia, and subsequent synthesis of amides. As a result of these changes, ammonia was maintained at a relatively low level.

TABLE II

AMIDE PRODUCTION IN STARVED LEAVES OF PEONY (MIYACHI)

NITROGEN	FRESH LEAVES	LEAVES STARVED 15 DAYS
	%	%
Total	1.364	1.462
Protein	1.312	0.801
Asparagine	0.037	0.206
Other amides	0.015	0.455
	PERCENTAGE OF TOTAL N	
Protein	96.19	54.79
Asparagine	2.71	14.09
Other amides	1.10	31.12

The formation of amides, therefore, should be considered as a "defensive mechanism" for the detoxication of NH_3, which comes into operation when there is a rapid destruction of proteins and accumulation of NH_3, or when there is an excessive reduction of NO_3 to NH_3.

Probably the most extensive and complete studies of nitrogen changes in leaves have been conducted by MOTHES (**14, 15**). After investigating a series of plants that had been subjected to various environments and experimental treatments, he draws the following conclusion: When leaves are exposed to light or are fed with glucose in darkness, no amides are formed and even the original amount disappears by being used in synthesis of proteins. But when the carbohydrate content of the leaves is reduced to a low point, as a result of long exposure to darkness, then amides are produced promptly and eventually ammonia appears. Oxygen seems to be a

TABLE III

CHANGES IN AMINO ACIDS AND AMIDES IN CURING TOBACCO LEAVES (VICKERY)

TIME	AMINO-N LOSS	AMIDE-N GAIN
hr.	*gm.*	*gm.*
41	0.020	-0.005
64	0.015	-0.017
87	0.370	0.019
111	0.007	0.018
159	0.110	0.278
183	0.395	0.318
207	0.290	0.449
231	0.515	0.546
279	0.820	0.835
303	0.290	0.446

limiting factor in amide formation. Without its presence, neither amides nor ammonia will be produced even in leaves of a low carbohydrate content, but there will be an increase of amino, basic, and other ("rest-N") nitrogen fractions.

Oxidation of the products of hydrolysis of proteins takes place in leaves when there is a shortage of carbohydrates, the final end product being ammonia. In presence of some carbohydrates, ammonia is "neutralized" by being used for the formation of asparagine, in which form NH_3 is "stored." Whether produced within the plant or supplied from an external source, asparagine will remain as such unless carbohydrates become available, when it enters promptly into synthesis of proteins. So, too, when ammonium salts are fed to leaves high in carbohydrates, proteins will be formed quickly; when carbohydrates are short, asparagine will be produced; and when carbohydrates are absent, ammonia will accumulate in the cells until poisoning of the leaves occurs. In this respect, ammonia supplied from without behaves physiologically the same as NH_3 released within the plant. When leaves with ample carbohydrate supply are exposed to narcotics, no amides and often no ammonia will be produced.

These conclusions are in striking agreement with the results of other investigators, as the succeeding discussion will show. MOTHES seems to have verified with mature plants most of the evidence obtained with seedlings.

3. SOME FACTORS AFFECTING ASPARAGINE FORMATION

While the procedure and mechanism of accumulation and disappearance of asparagine and related substances in plant metabolism are not yet understood in all their details, we know that several factors seem to affect them. Some of these will be discussed briefly.

a. IMPORTANCE OF OXIDATION.—Proteins may be decomposed in plants with or without the presence of oxygen, the usual hydrolysis products being the amino acids (**19**). In the presence of oxygen, however, asparagine (or glutamine) often appears as the main soluble N substance. With further oxidation it is broken down to NH_3. That oxidation is really essential for the production of amides and ammonia has been demonstrated, among others, by SUSUKI (**49**) with barley (table IV) and soy beans, and by BUTKEWITSCH (**3**) with lupine (table V). SUSUKI found not only an increase in asparagine when seedlings were developed in darkness in presence of oxygen, but also a marked decrease of amino acids. This has been corroborated by WASSILIEFF (**53**). Without a supply of oxygen, amides are not formed and amino acids accumulate. With further oxidation and absence of carbohydrates, ammonia is produced in large quantities owing to the breakdown (oxidation) of asparagine (table V). SURE and TOTTING-

TABLE IV

EFFECT OF O_2 ON ASPARAGINE FORMATION IN BARLEY SEEDLINGS (SUSUKI)

	DRY MATTER	PER 100 SEEDLINGS
	%	*gm.*
At beginning of experiment	6.59	0.1427
After 45 hours in darkness with O_2	8.85	0.1818
After 45 hours in darkness without O_2	7.16	0.1336

TABLE V

EFFECT OF O_2 ON FORMATION OF AMMONIA AND AMIDE-N IN GERMINATING SEEDS OF LUPINE (BUTKEWITSCH)

NITROGEN	PER 100 SEEDLINGS	
	WITHOUT O_2	WITH O_2
	mg.	*mg.*
Ammonia	19.38	170.60
Amide	171.36	139.48
Ammonia and amide	190.74	310.08

HAM (**47**) have shown also that in the shoots of pea seedlings there is a simultaneous decrease of α-amino acids and ammonia, which indicates that "α-amino acids serve for amide production in the nitrogen metabolism of the etiolated pea plant."

b. EFFECTS OF ANAESTHETICS.—Since the time when CLAUDE BERNARD (1878) demonstrated that anaesthetics inhibit anabolism but permit catabolism, various experiments have been performed to show their effects on N metabolism in plants. BUTKEWITSCH (**3**), for example, exposed seedlings to fumes of toluol with the result that no asparagine was formed but instead large amounts of NH_3 accumulated, up to 14 per cent. of total N. This suggests that asparagine arises not as a result of tearing down but through a building up or synthetic process.

c. EFFECTS OF PRESENCE OF CARBOHYDRATES.—It has already been noted that with extreme carbohydrate deficiency the carbon structure of the asparagine molecule is broken down (oxidized) and NH_3 accumulates. Synthesis of asparagine may be effected by means of an external supply of glucose, as table VI shows. An artificial supply of sugar will not only diminish the accumulation of NH_3 but will result in a marked increase of amides (asparagine) (**3, 46**), which eventually will lead to the formation of proteins (**49, 46**). Several investigators have found that asparagine disappears when seedlings, grown in darkness, are supplied with sugar

TABLE VI

EFFECT OF GLUCOSE ON AMMONIA AND AMIDE-N CONCENTRATION IN SEEDLINGS OF LUPINE (BUTKEWITSCH)

NITROGEN	WITHOUT GLUCOSE	WITH GLUCOSE
	%	%
Ammonia	18.57	9.37
Amide	15.17	23.10

(**48, 46**). Contrariwise, an abundance of sugar prevents asparagine formation from proteins, but under the right conditions it does not interfere with and may even stimulate the synthesis of this amide.

One may conclude with PRIANISCHNIKOV (**26**), therefore, that with carbohydrate shortage, proteins will be hydrolyzed and there will be formed amino acids of the general formula $R(NH_2)COOH$, which, through oxidation and secondary synthesis, produce acid amides of the general formula $R(NH_2)CONH_2$ (asparagine and glutamine), and, in case of carbohydrate starvation, give, through further oxidation, NH_3. But when the carbohydrate supply is abundant, whatever its source, then the reverse process takes place. From NH_3 to acid amides ($R(NH_2)CONH_2$), which, with additional supply of carbohydrates, give rise to amino acids ($R(NH_2)$-$COOH$), and these in turn synthesize proteins.

The frequently observed results of formation of asparagine in light and not in darkness are due not to the direct effect of light but to the presence of carbohydrates in light, as was demonstrated by PRIANISCHNIKOV (**28**), who gives the schematic summary shown in table VII.

d. SYNTHESIS OF ASPARAGINE FROM AMMONIUM SALTS.—One of the crucial experiments in the determination whether asparagine originates directly from breakdown of proteins or comes from the synthesis of NH_3 with other oxidation products of proteins, is through feeding plants in various states of carbohydrate deficiency with ammonium salts.

TABLE VII

CARBOHYDRATE AND LIGHT RELATIONSHIPS IN THE FORMATION OF ASPARAGINE AND AMMONIA (PRIANISCHNIKOV)

EXPERIMENTAL CONDITIONS		RESULTS	
CARBOHYDRATES	LIGHT	ASPARAGINE SYNTHESIS	AMMONIA INJURY
+	−	+	−
−	−	−	+
+	+	+	−
−	+	−	+

Ever since SUSUKI (48), HANSTEEN (7), and ZALESKI (55) demonstrated that proteins may be formed from various nitrogenous substances supplied to the plants, much work has been done with the object of determining to what extent and under what circumstances asparagine is synthesized from N constituents of nutrient solutions. Thus PRIANISCHNIKOV (**24, 25, 26, 29**) and others (**48, 46**) have shown that by supplying salts of nitrogen to certain seedlings their asparagine content is markedly increased. For this purpose ammonia is a better source of N than the nitrates, when the physiological acidity of ammonium salts is neutralized by a base ($CaCO_3$, etc.) (**48, 25**). The legumes in particular seem to require Ca to neutralize the acid reaction of NH_4Cl and $(NH_4)_2SO_4$, which appear to inhibit the formation of asparagine (table VIII). Barley and corn seedlings, high

TABLE VIII

RESULTS OF FEEDING VETCH SEEDLINGS WITH AMMONIUM SALTS WITH AND WITHOUT CALCIUM (PRIANISCHNIKOV)

AMOUNT N IN 100 SEEDLINGS	DISTILLED H_2O	NH_4Cl	$NH_4Cl + CaCO_3$
	mg.	*mg.*	*mg.*
Protein N	85	109	90
Asparagine N	75.9	73.9	118.2
Ammonia N	0.9	0.9	1.0

in carbohydrates, will absorb NH_3 from these salts equally well either with or without the presence of calcium. But when carbohydrates are exhausted (darkness) in corn or barley seedlings, they will behave like legumes. And legumes can be put, by various methods, into a physiological state similar to that of cereal seedlings (table IX.)

TABLE IX

RELATION OF NITROGEN TO CARBOHYDRATES IN VARIOUS TYPES OF SEEDLINGS (PRIANISCHNIKOV)

GRAMINEAE	STARCH CONTAINING LEGUMES	LUPINE
1: 6	1: 2.0 – 2.5	1: 0.6

The importance of Ca ions in this respect is not clearly understood, for this ion increases even the utilization of N from nitrates (**25**). Very likely calcium has something to do with the carbohydrate metabolism. It of course neutralizes to some extent the acidity of the nutrient medium, since cations (NH_4) are absorbed faster than anions. PRIANISCHNIKOV thinks that Ca ions increase respiration and hydrolysis of proteins.

Lupine seedlings with very small carbohydrate reserves are unable to assimilate N from either NH_4Cl or $(NH_4)_2SO_4$, even in presence of Ca salts. But when exposed to light or fed glucose, they will utilize N under the above conditions. Similarly barley seedlings, after prolonged growth in darkness and loss of all starch reserves, are incapable of assimilating nitrogen. When in this state they behave like lupine seedlings. Carbohydrates and the essential internal environmental factors, therefore, are necessary for the synthesis of asparagine from inorganic nitrogen.

e. Synthesis of asparagine from NH_3 and organic acids.—Because of the similarity in chemical structure between some of the organic acids and acid amides, it has long been suspected that they may be interrelated. Moreover, evidence has accumulated in support of the view that organic acids are produced when proteins are broken down (**12**, **34**, **35**), although very likely they may be formed also from sugars (**14**). Malic and succinic acids seem to be the two that, in presence of ammonia, participate readily in the synthesis of asparagine. By feeding corn seedlings ammonium sulphate and ammonium salts of organic acids, Smirnov (**46**) was able to demonstrate that both malic and succinic acids enter into the synthesis of asparagine (table X), and that, in presence of glucose, eventually proteins accumulate (table XI).

TABLE X

Results of feeding corn seedlings ammonia with and without organic acids (Smirnov)

Form of ammonia	Percentage of total N			
	Protein N	Ammonia N	Asparagine N	Amino acid N
	%	%	%	%
$(NH_4)_2SO_4$	66.62	4.19	13.34	15.85
Ammonium malate	58.42	3.87	19.30	18.41
Ammonium succinate	63.57	3.33	16.16	16.94

TABLE XI

Protein synthesis in corn from asparagine in presence of glucose (Smirnov)

Source of ammonia	Percentage of total N		
	Protein N	Ammonia N	Asparagine N
	%	%	%
$(NH_4)_2SO_4$	66.79	4.4	15.10
Ammonium succinate	67.75	4.2	19.52
Ammonium aspartate	70.25	4.5	17.6

Many investigators (PRIANISCHNIKOV etc.) conceive the path of formation of asparagine from malic acid through aspartic acid and ammonium aspartate (table XII).

TABLE XII

ANALOGY BETWEEN ASPARAGINE AND UREA

Probable formation of asparagine in plants:

$COOH$		$COOH$		$COOH$		$COOH$
$CHOH$		$CHNH_2$		$CHNH_2$		$CHNH_2$
CH_2	$\longrightarrow$	CH_2	$\longrightarrow$	CH_2	$\longrightarrow$	CH_2
$COOH$		$COOH$		$COONH_4$		$CONH_2$
malic acid		aspartic acid		ammonium aspartate		asparagine

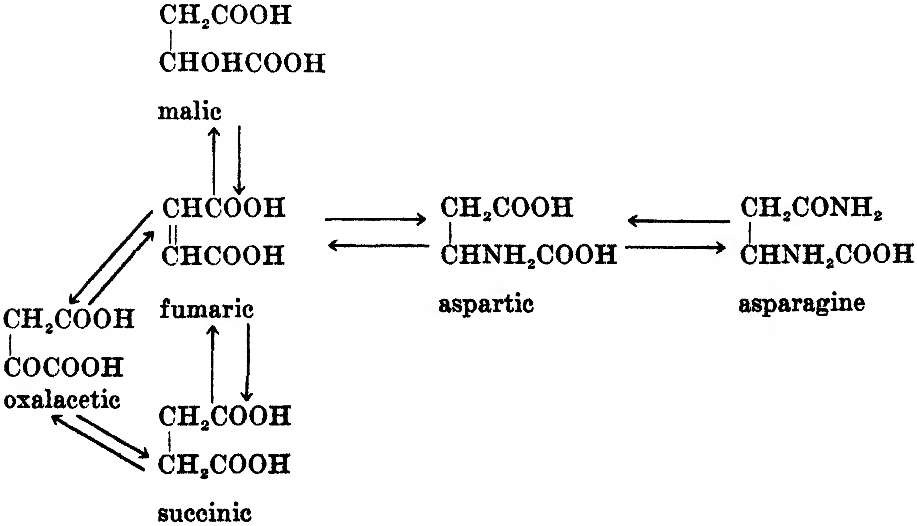
In animals:

$HOCOOH$	$\longrightarrow$	NH_2COOH	$\longrightarrow$	NH_2COONH_4	$\longrightarrow$	NH_2CONH_2
carbonic acid		form-amino acid		ammonium carbamate		urea

Based on ROBINSON'S (33) ideas, VICKERY and PUCHER (52) present an additional plan, which shows the connection between asparagine and α-keto and hydroxy acids (table XIII). They state that "this purely hypothetical scheme involves, however, only reactions of known biological significance."

TABLE XIII

ROBINSON'S SCHEME SHOWING CONNECTION BETWEEN ASPARAGINE AND α-KETO AND HYDROXY ACIDS (VICKERY AND PUCHER)



Another more direct proof that ammonium salts of organic acids, although participating in the synthesis of proteins, are not so easily assimilated as asparagine, comes from experiments of feeding plants with these substances. NAKAMURA (**16, 17**), for instance, found that both phanerogams and fungi utilize more readily asparagine than ammonium succinate from a weak nutrient solution. By feeding asparagine to leaves of high carbohydrate content, PRIANISCHNIKOV and SMIRNOV were able to demonstrate protein synthesis. When carbohydrates were absent, asparagine remained intact in the leaves. This has been corroborated by NAKAMURA with barley seedlings. Hence there seems to be considerable proof in support of the above path of utilization of asparagine in protein formation.

f. ORGANIC ACIDS AS DIRECT RECEPTORS OF AMMONIA.—Recently RUHLAND and WETZEL (**34, 35**) have shown that in some highly acid plants (*Oxalis, Begonia, Rheum*) oxalic acid takes care of ammonia by forming ammonium oxalate and possibly ammonium salts of other acids. Amides are not present in any appreciable quantities in these plants, excepting in the older leaves, which are less acid. In *Rheum* both amides and organic ammonium salts may serve as receptors of NH_3 in various organs of the same plant, depending on their relative acidity. They conclude, therefore, that amides are formed in less acid plants (or tissues) and organic salts of ammonia in more acid ones.

There is much information on record showing a connection between organic acids and protein transformation. Malic, tartaric, citric, and other acids may come either from amino acids or from intermediary products of protein disintegration. But a strongly oxidized acid, like oxalic, can be derived from sugars as well as proteins.

Continuing RUHLAND and WETZEL's investigations, KULTZSCHER (**13**) found that very acid plants ($pH < 5.00$) are able to store large quantities of excess nitrogen in the form of ammonium salts of organic acids. Their function is not merely a case of neutralization of NH_3 but also of storage of N. In deamination of amino acids not only NH_3 is released but also acids are formed, which then more or less automatically take care of each other by forming ammonium salts.

According to KULTZSCHER, it would seem that an equilibrium exists between amides and ammonia. In plants relatively high in actual (pH) and potential acidity, the equilibrium is shifted to the NH_3 side and ammonium salts are formed through the union of NH_3 with organic acids. The fact is emphasized by KULTZSCHER that highly acid plants are characterized by an active deamination process.

Ammonium salts of oxalic and other organic acids will take care of proportionally larger quantities of NH_3 than asparagine does, but urea of

still more, and guanidine, $\begin{array}{l} H_2N \diagdown \\ \qquad C\text{–}NH_2 \\ HN \diagup\!\!\diagup \end{array}$, most of all the compounds so far found in plants. The ratios of carbon to nitrogen in various substances that may act as receptors of nitrogen are presented in table XIV.

TABLE XIV

RATIO OF CARBON TO NITROGEN IN VARIOUS ORGANIC SUBSTANCES WHICH MAY ACT AS NITROGEN RECEPTORS IN PLANTS

	C : N
Glutamine	2.5 : 1
Asparagine	2 : 1
Ammonium malate or tartrate or succinate or citrate	2 : 1
Arginine	1.5 : 1
Ammonium oxalate	1 : 1
Allantoin	1 : 1
Urea	1 : 2
Guanidine	1 : 3

g. FORMATION OF UREA AND OTHER MEANS OF REMOVING NH_3.—Many fungi are capable of forming and even absorbing from without large quantities of urea, $CO(NH_2)_2$, but as a rule do not excrete it. According to IWANOV (**8**) some fungi (*Lycoperdon*, *Bovista*) may accumulate half of the total N as urea (up to 11 per cent. of dry weight). Fungi will absorb urea and thiourea from weak solutions of these substances and store it up to 15 per cent. of their dry weight (**9**).

Urea in plants may come also from the breaking down of arginine,

$$\begin{array}{l} H_2N \diagdown \quad H \qquad\qquad\qquad\qquad NH_2 \\ \qquad C - N - CH_2 \cdot CH_2 \cdot CH_2 \cdot \overset{\diagup}{C}H \cdot COOH \\ HN \diagup\!\!\diagup \end{array}$$

which itself may act as an NH_3 receptor. Ammonia is split off easily from arginine by the enzyme arginase (**11**). Arginine is supposed to have the same function in conifers as asparagine in other plants.

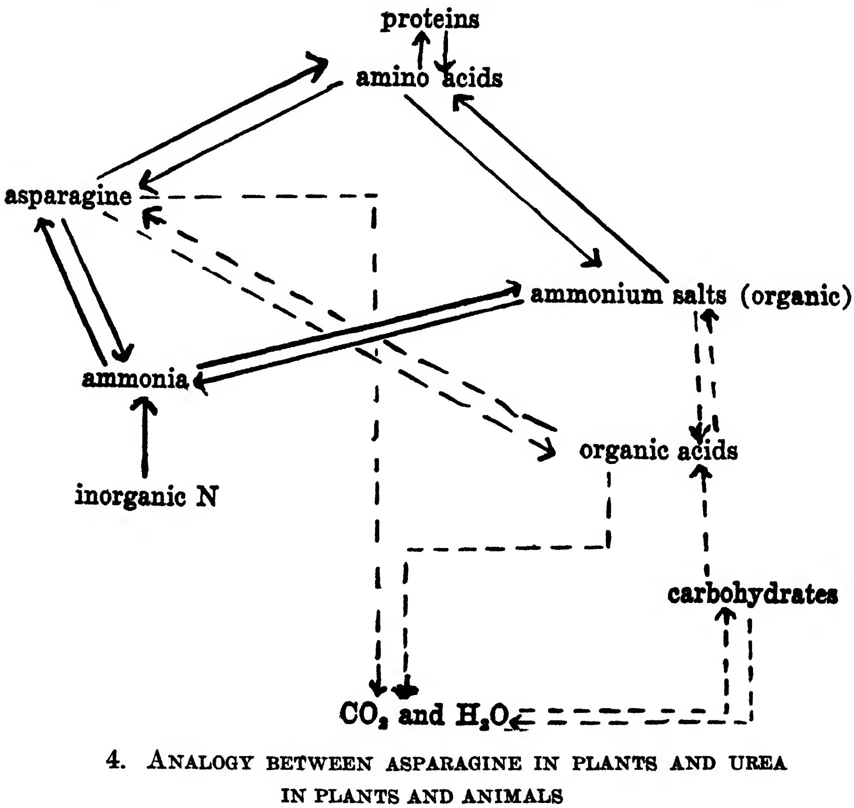
Whatever its source, urea seems to accumulate in some plants in absence of carbohydrates (**10**), and since it is used up in certain stages of the development of the organism, it should be considered as a storage form of N, and therefore analogous to asparagine and glutamine in its physiological function. Moreover, urea may be changed to asparagine. According to SUSUKI (**48**) and PRIANISCHNIKOV (**25**), it is frequently a better nutrient form of N than ammonium salts for the formation of asparagine.

From the preceding discussion it is apparent that there are at the disposal of plants various means of removing and neutralizing NH_3. The mechanism set into operation in each instance may possibly depend upon several factors, of which the most important seems to be the carbohydrate content of the plant as a whole or of specific organs, the hydrogen ion concentration and the source (meaning the rest of the molecule) from which NH_3 comes. Doubtless many environmental factors also play a rôle in this respect. Still one must be mindful of the fact that under extreme conditions of carbohydrate depletion, nitrogen may be excreted from the plant through the roots in the form of ammonia (**30, 31**).

A generalized scheme of nitrogen metabolism in plants, modified after Engel (**6**), is presented in table XV.

TABLE XV

Scheme of N metabolism in higher plants (modified after Engel)



4. Analogy between asparagine in plants and urea in plants and animals

Both amides, asparagine and urea, do not seem to be the direct products of protein hydrolysis but arise from secondary synthesis with NH_3 as the

key ion, which most often comes from oxidation of amino acids. Both amides may be synthesized in plants when NH_3 is introduced into the organism, with this difference between the two, that for synthesis of asparagine a part of the unoxidized carbohydrate molecule is necessary while for formation of urea NH_3 and CO_2 are sufficient (table XII). The physiological function of both processes appears to be the neutralization and storage of NH_3, which seems to be toxic to living organisms.

The analogy between plants and animals in this respect is that neither of them can synthesize the respective amides from ammonium salts of strong acids, but more neutral salts of ammonia will lead to such synthesis.

There is a great difference, however, in respect to the further metabolic rôle of the two amides. Urea is excreted from animals. They do not need to be so economical with N, for its intake through feeds is more or less assured and there is no caloric value in CO_2, the carbon part of the molecule. Asparagine, on the other hand, remains in the cells of plants as a reserve substance of N, which, with renewed supply of carbohydrates, can be used again for the production of amino acids and other components of proteins.

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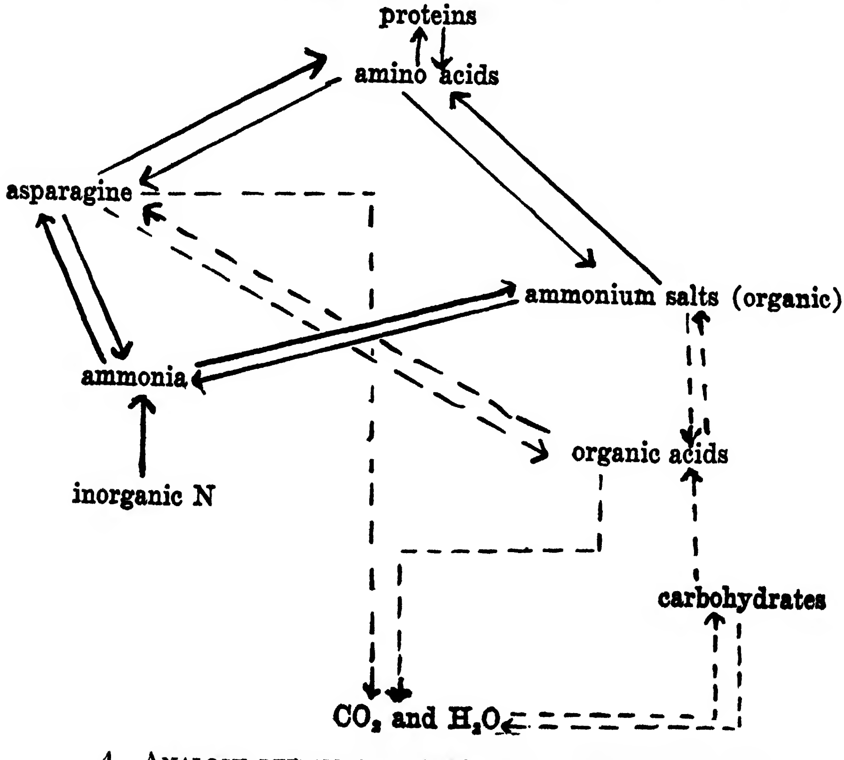
From the preceding discussion it is apparent that there are at the disposal of plants various means of removing and neutralizing NH_3. The mechanism set into operation in each instance may possibly depend upon several factors, of which the most important seems to be the carbohydrate content of the plant as a whole or of specific organs, the hydrogen ion concentration and the source (meaning the rest of the molecule) from which NH_3 comes. Doubtless many environmental factors also play a rôle in this respect. Still one must be mindful of the fact that under extreme conditions of carbohydrate depletion, nitrogen may be excreted from the plant through the roots in the form of ammonia (**30, 31**).

A generalized scheme of nitrogen metabolism in plants, modified after ENGEL (**6**), is presented in table XV.

TABLE XV

SCHEME OF N METABOLISM IN HIGHER PLANTS (MODIFIED AFTER ENGEL)



4. ANALOGY BETWEEN ASPARAGINE IN PLANTS AND UREA IN PLANTS AND ANIMALS

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FACTORS INFLUENCING THE GROWTH AND YIELD OF POTATOES IN FLORIDA

M. R. Ensign

(WITH NINE FIGURES)

Introduction

It is common knowledge that the early Irish potato crop in Florida shows wide fluctuations in yields from year to year; plantings made at various times during any one year also show as erratic variations. It is the consensus of opinion among Hastings and LaCrosse growers that the plantings made after the middle of January have usually produced larger average yields than those planted prior to that time. At present, the time of planting in the Hastings area[1] begins about the first part of December and extends to the first week in February. Plantings at LaCrosse usually do not begin prior to January 10.

A series of time-of-planting tests was begun in 1927 and continued until the harvesting of the 1931 crop. The object of these experiments was to determine, if possible, some of the factors influencing the growth and yield of potatoes.

Review of literature

The literature dealing with potato experiments shows that rainfall (soil moisture) and temperature exert considerable influence upon growth and yield. The irrigation experiments of Widtsoe (8, 9, 10) and Harris (4, 5) of Utah were especially significant since similar results were obtained from repetitions extending over two 5-year intervals. Their results showed that the middle period of growth, about the time of blossoming and tuber setting, was the most sensitive to moisture variations as reflected by the yield; if but one irrigation was given during the growing season, the best results were obtained when this was applied during the middle period of growth. Smith (7) obtained similar results when he compared the rainfall with the potato yields in Ohio over a 50-year period. He too found a 30–40-day period about the time of blossoming to be the most sensitive to water requirements.

The work of Bushnell (1) showed that as temperatures rose above 68° F. (20° C.) the yield of potatoes decreased, and when grown at a temperature of 86° F. practically no tubers were formed, since respiration was so rapid as to consume the food as soon as it was manufactured. Hardenburg (3)

[1] Federal Point is included in the Hastings area, and the earliest plantings begin there, since it enjoys some frost protection on account of its proximity to the St. Johns River.

grew potatoes in two greenhouses, in one of which he maintained an approximate temperature of 60° F. while the other was operated at a temperature 8° higher. The larger yield was produced at the lower temperature.

Numerous other investigations corroborate these essential facts, namely: (1) soil moisture, measured in inches of rainfall or acre inches of irrigation, plays an important part in the growth and yield of potatoes, and yields are most noticeably influenced by variations in available water during the middle period of growth; (2) temperatures above 68° F. during the growing period restrict the yield of potatoes.

Methods of experimentation

The time-of-planting tests were begun on January 10, 1927, and continued for five consecutive years. Plantings were made only at LaCrosse the first year; at Hastings, as well as LaCrosse, the second year; and at Hastings only the last three years. The tests at LaCrosse were discontinued after 1928 because of wide soil variations and because of the presence of a soil-borne disease, *Rhizoctonia,* that interfered with the interpretation of results.

Plantings were made at weekly intervals the first two years, beginning January 10 and ending February 20. It then became evident that the planting range should be extended to include two other periods, mid-December and January 1. These dates were therefore included in the planting schedule of the last three years of the experiment.

The prevailing soil type on which the tests were conducted both at LaCrosse and Hastings is commonly called flatwoods, and is classified as Bladen, consisting of a sandy loam with a clay subsoil 12–18 inches below the surface which tends to insure a fairly satisfactory and constant soil moisture content. The water saturation point of these soils was slightly above 22 per cent.; the wilting point about 1 per cent., based upon oven dryness of samples from the first foot of soil; the average pH was 4.78. The fields used for the tests had been almost continuously cropped to potatoes for ten years, with corn and cowpeas or peanuts as summer crops.

A ready-mixed fertilizer made up of 5 per cent. available nitrogen, 7 per cent. phosphoric acid, and 5 per cent. potash was distributed at the rate of 2000 pounds per acre in the drill two weeks prior to planting each lot of seed.

Only Maine certified seed of the Spaulding Rose variety was used, the same source being adhered to in all the plots each year. The seed was cut by hand the day prior to planting and the pieces were made as uniform in size as practicable, averaging about 1.5 oz. each. Copper lime dust was applied at 7–10-day intervals after the plants were 4–6 inches high, to combat disease. Late Blight was the most serious disease in the Hastings area,

although Early Blight was not altogether absent. *Rhizoctonia* and Early Blight were prevalent in the LaCrosse section in certain years. Such diseases are very disturbing in time-of-planting tests, since blight epidemics spread readily from older to younger plantings in adjoining plots in spite of vigorous control measures. Notations were made of the time of initial infection, the progressive spread and the relative damage to foliage of the potatoes of each planting, and these factors were given due consideration in the final interpretation of results, but no correction of data was attempted.

Continuous thermographic soil temperature records were made during the growing season at the depth of the seed-piece (approximately 2.5 inches deep at Hastings and somewhat deeper at LaCrosse). Soil moisture determinations were made at weekly intervals during the growing period from each plot by taking composite samples with a soil tube to a depth of 1 foot, measured from the top of the ridge. The percentage of soil moisture was computed on an oven-dry basis.

The time of harvesting each planting was determined, to some extent, by the weather and by the farmer-cooperator. In general, from 90 to 100 days were allowed from planting date to harvest. Individual hill records were taken of 75 to 150 hills from a number of replications. There were 8–12 replications per planting. These records included total number and weight of tubers per hill, number of 1's and 2's, and their respective weights. The remaining hills of each replication were combined so that total weights and total number of tubers were secured for each replication. The yield in barrels[2] per acre of United States grade no. 1's, or prime tubers, is used in this study as a measure of the most effective time of planting. The relative yield of prime potatoes per acre is governed by the number of such tubers per hill and by their size or weight. Some consideration has also been given to the yield of no. 2's, even though any planting having a large proportion of this grade of tubers may be regarded as having been produced under unfavorable environmental conditions.

Weights of tubers were recorded to the nearest gram, using a wire basket as a container, which facilitated shaking out any loose dirt prior to weighing. In most cases the tubers from the sandy soil were relatively clean when dug, although it was necessary to wash some of them.

In converting the yield in grams per plot to barrels per acre, a constant number of hills per acre, 10,700, was assumed, based upon rows 40 inches apart and hills spaced 14.5 inches in the row. Then by the use of a constant derived from the fraction in the formula, $Y \times \frac{1}{74{,}844.A}$ barrels per acre, comparable results throughout the tests were assured. In this for-

[2] A barrel contains 11 pecks or 165 pounds of potatoes.

TABLE I

AVERAGE YIELDS PER ACRE OF PRIME POTATOES AT LACROSSE AND HASTINGS, FROM 1926 TO 1931

YEAR AND PLACE	DATE OF PLANTING								ANNUAL MEAN
	DEC. 15	JAN. 1	JAN. 15	JAN. 21	JAN. 28	FEB. 4	FEB. 11	FEB. 20	
	bbl.	*bbl.*	*bbl.*	*bbl.*	*bbl.*	*bbl.*	*bbl.*	*bbl.*	
LaCrosse 1926–27			35.01	33.80	8.20	34.00	46.10	51.00	34.69
LaCrosse 1927–28 ..			60.05	73.35	60.60	69.75	64.15	52.30	63.37
Hastings 1927–28 ...			.. .	46.00	47.95	52.58	46.88	37.30	46.14
1928–29 .	32.86	20.01	34.94	40.26	13.18	5.47	3.67		21.48
1929–30 . .	30.19	23.67	18.69	26.63	19.35	13.99	7.16	8.19	18.48
1930–31 ...	16.76	40.38	39.13	26.17	28.15	17.32	10.74	5.83	23.06
Mean (Hastings only)	26.60	28.02	30.92	34.77	27.16	22.34	17.11	17.11	....

* Average probable error for all plantings = ± 0.74 barrel per acre.

mula Y represents the yield in grams per plot of area A, while 74,844 is the number of grams in a barrel of potatoes weighing 165 pounds net.

Experimental results

I. Yields of no. 1's and no. 2's

The yields of United States grade no. 1 potatoes in barrels per acre are shown in table I for the various plantings at LaCrosse and Hastings during the 5-year period from 1927 to 1931 inclusive.

Table I shows two facts of particular interest: (1) The wide variation in yield of no. 1's secured from plantings on the same date[3] but in different years; and (2) the uniformity of the mean yield over a considerable part of the planting season tested. Maximum yields were not secured twice for any one planting date. In 1928, the February 4 planting was best, while the largest yields came from the January 21, December 15, and January 1 plantings in the successive years. Furthermore, the growing conditions in 1928 were favorable throughout the whole planting range[4] as compared with

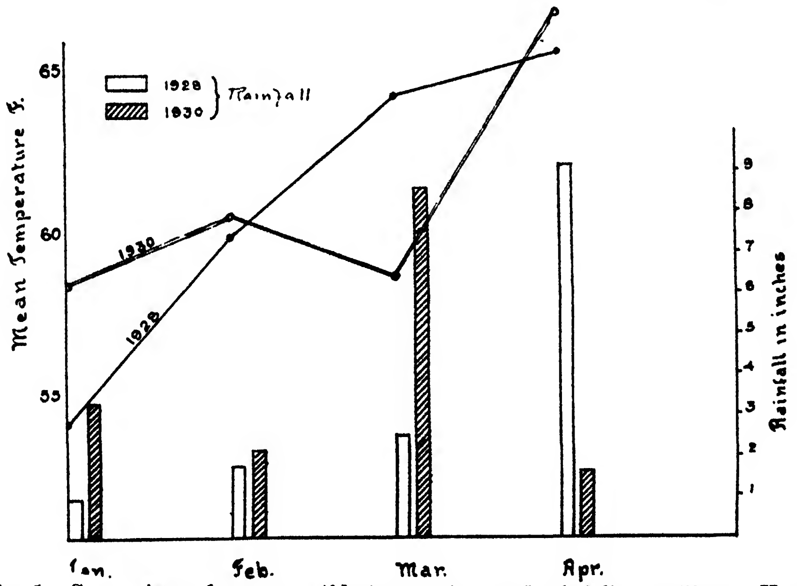

FIG. 1. Comparison of mean monthly temperature and rainfall prevailing at Hastings during the growing seasons of 1927–28 and 1929–30. The former was characterized by large and the latter by small yields. The weather conditions obtaining during February of these years were not essentially different, but the wide divergences for the other months, especially that of March, should be noted.

[3] It was impractical to plant on exactly the same date each year but the variation was not more than three days, so that the nearest average date of planting is shown in the tables and graphs.

[4] Early commercial plantings in the same fields with the experimental plots produced good yields.

TABLE II

Proportion of prime, no. 2's, and cull potatoes by weight, from LaCrosse and Hastings, 1927–28 to 1930–31

Year and place	Date of planting											
	Dec. 15			Jan. 1			Jan. 15			Jan. 21		
	1's	2's	Culls	1's	2's	Culls	1's	2's	Culls	1's	2's	Culls
	%	%	%	%	%	%	%	%	%	%	%	%
LaCrosse												
1927–28					.		70.4	19.6	10.0	80.4	13.2	6.4
Hastings												
1927–28								..	.	68.7	23.1	8.2
1928–29	58.8	28.2	13.0	47.5	41.2	11.3	61.5	27.1	11.4	61.8	31.8	6.4
1929–30	53.6	24.2	22.1	52.3	23.8	23.9	44.0	30.0	26.0	49.8	26.7	23.5
1930–31	40.6	29.1	30.3	59.2	24.3	16.5	63.3	22.7	14.0	45.9	30.5	23.6
Mean	51.0	27.2	21.8	53.0	29.8	17.2	59.8	24.8	15.3	61.3	25.1	13.6
Mean for Hastings only	51.0	27.2	21.8	53.0	29.8	17.2	56.2	26.6	17.1	56.5	28.0	15.4

TABLE II—(*Continued*)

Year and Place	Date of planting											
	Jan. 28			Feb. 4			Feb. 11			Feb. 20		
	1's	2's	Culls	1's	2's	Culls	1's	2's	Culls	1's	2's	Culls
	%	%	%	%	%	%	%	%	%	%	%	%
LaCrosse												
1927–28	76.5	15.4	8.1	79.0	12.9	8.1	75.5	15.0	9.5	67.6	18.2	14.2
Hastings												
1927–28	62.8	19.4	17.8	72.6	20.6	6.8	63.8	26.1	10.1	59.7	28.6	11.7
1928–29	32.9	44.9	22.2	16.3	52.1	31.6	11.8	46.9	41.3	...	...	...
1929–30	38.2	31.5	30.3	37.3	28.3	34.4	25.9	29.2	44.9	30.0	31.9	38.1
1930–31	53.3	32.1	14.6	42.6	32.7	24.7	37.3	32.3	30.4	31.8	33.9	34.3
Mean	52.7	28.6	18.6	49.6	29.3	21.1	42.9	29.9	27.2	47.5	28.2	24.6
Mean for Hastings only	46.8	31.9	21.2	42.2	33.4	24.4	34.7	33.6	31.7	40.5	31.5	28.0

those prevailing in 1929–30. These facts indicate that certain climatic factors in specific years are not only more conducive to high yields (fig. 1), but that at certain times during the normal growing period of each year there are more favorable relations than at others. The relatively dry cold January and the dry warm March of 1928 seem to be significant as related to high yields of prime tubers. A comparison of other high and low yielding years confirms this relationship.

The production of a large percentage of no. 2 potatoes is undesirable since strong competition on the markets has made it increasingly difficult to sell this grade of tubers advantageously. For this reason the yield data contained in table II are interesting.

Table II shows that one-fourth to one-third of the total weight of tubers produced during the 5-year period were no. 2's, and that as a rule the larger proportion of this grade was secured from the latest plantings. This was especially noticeable in the last three plantings in the season of 1928–29. There are two explanations for this condition: (1) Late Blight often killed immature vines, thus arresting the growth of tubers that would otherwise have become no. 1's; and (2) the later plantings were harvested from 78 to 90 days after planting in contrast to 100 to 110 days for the earlier plantings. The large proportion of culls (no. 3's and 4's) in many of the plantings should also be noted, for these represent waste as a general rule. The highest percentage of no. 1's, as well as the largest yields, were secured on the average from the January 15 and January 21 plantings.

II. Effects of rainfall and soil moisture on yield and grade of tubers

A mathematical study of the relationship between yield of no. 1 tubers per acre[5] and rainfall by months during the potato growing season at Hastings for a 10-year period shows that relatively dry Marches and wet Aprils are conducive to high yields.[6] The experimental plantings covering the 5-year period also confirm this fact.

To determine the relative importance of soil moisture at any particular stage of potato development upon the yield of no. 1 tubers, four stages of growth were studied as follows: (1) the period of germination, *i.e.*, from the date of planting until a majority of the sprouts were above ground,—usually from 20 to 24 days after planting; (2) the period of stolon formation, which usually begins in the faster growing hills about the 28th day

[5] Average yield data for the entire area at Hastings were furnished through the courtesy of the Hastings Potato Association.

[6] The following correlation coefficients were derived:

December 0.091 ± 0.18; January -0.231 ± 0.20; February 0.063 ± 0.16;

March -0.77 ± 0.087; April 0.65 ± 0.12; entire growing period -0.12 ± 0.20.

It was also shown that wet Marches have a tendency to be cold, the correlation coefficient being -0.40 ± 0.07.

after planting and continues through the 7th week or 50th day in the slower-growing plants; (3) the tuber-setting and tuber-growing period, which begins immediately after stolon formation and extends until harvest; (4) the entire growing period from planting until harvest.

Repeated observations in the field showed that the interval required for stolon formation of any one plant is from 7 to 10 days, and in any planting of 200 or more hills the process will reach its maximum about 35 to 40 days after planting, depending upon the growing conditions. Different varieties show some variation in the time of stolon formation; the data given are for the Spaulding Rose variety.

The partial correlation studies noted above indicated a distinct relationship between the yield of no. 1 tubers and the percentage of soil moisture during the period of stolon formation (28–50 days after planting). No well defined relationship appeared for the other growth periods noted. The yield-moisture relationship is shown in figures 2 and 3 for LaCrosse and Hastings respectively, for the season of 1927–28. The average soil moisture for the 28th to 50th days after planting was used as a basis of comparison with yields from the respective plots. In figure 2 the average soil moisture and the respective yields were plotted for each plot in all the plantings, while in figure 3 only the averages of all the plots for each planting were plotted against the average yield for that planting.

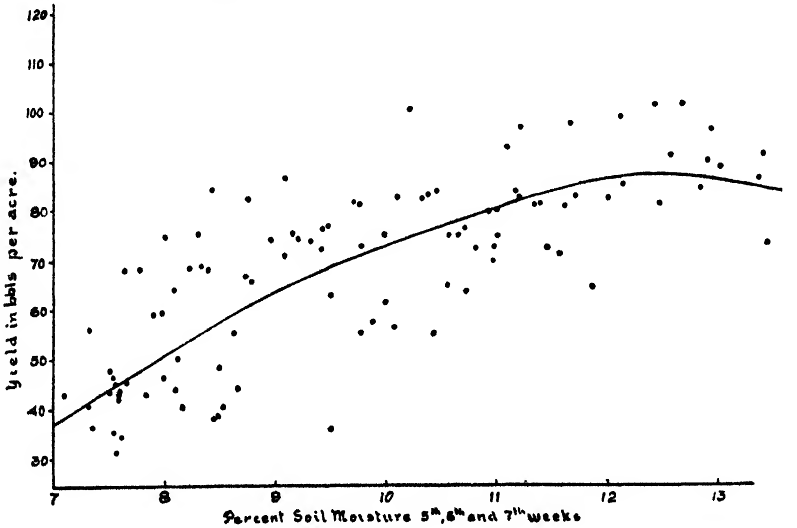

FIG. 2. Correlation between average soil moisture content during the stolon formation period and yield of no. 1 tubers in barrels per acre at LaCrosse, 1928. The curve was derived from a treatment of the data by the method of least squares. Note tendency toward an inverse relationship as the soil moisture rises above 12 per cent.

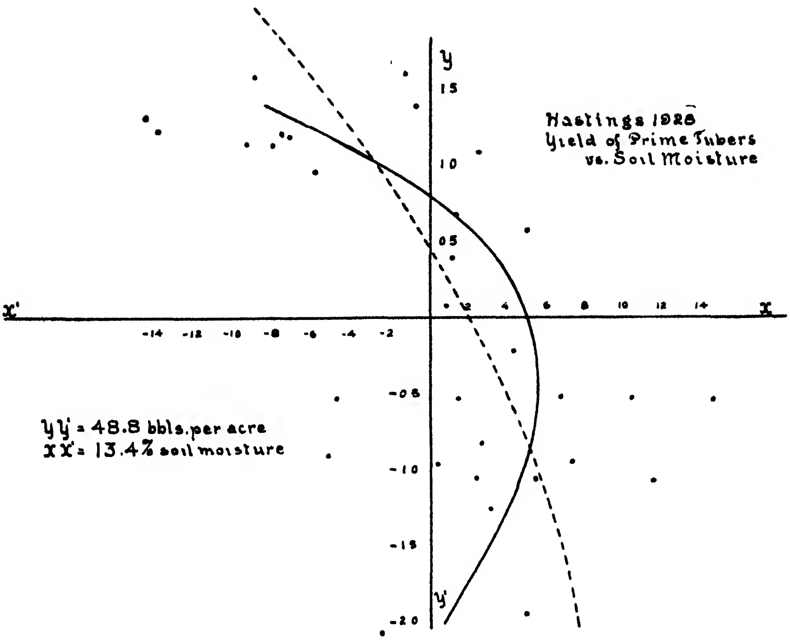

FIG. 3. Correlation between average soil moisture during the stolon formation period and yield of no. 1 tubers in barrels per acre at Hastings, 1928. Dotted curve was derived from a treatment of the data by the method of least squares, while the solid curve represents an interpretation of the data based upon field notes. The latter, likewise, more nearly fits the facts as derived from the other years of the investigation, especially with respect to the point of optimum moisture (about 12.5 per cent.). This is in reality a continuation of the curve shown in figure 2, showing inverse moisture-yield relationship.

The correlation shown in figure 2 (0.806 ± 0.023) is large and positive; that is, the yield of tubers at LaCrosse *increased* as the percentage of soil moisture *increased* during the stolon formation period. As the percentage of soil moisture went above 12, however, there was a distinct tendency for a reduction in yield.

The soil moisture content is considerably higher in all plots at Hastings than at LaCrosse over the same period of time, as a comparison of figures 2 and 3 will show. The range at LaCrosse was from 7 to 13 per cent., while at Hastings it ranged from 11.5 to 15.3 per cent. Therefore the correlation (-0.743 ± 0.18) shown in figure 3 is seen to be negative in contrast to that shown in figure 2; *i.e.,* the yields of prime tubers at Hastings *decreased* as the soil moisture *increased* during the stolon formation period. The most favorable moisture, however, appears to lie near 12 per cent., the same as at LaCrosse. The variation of this apparent optimum from year to year was not marked. In 1928–29 the largest

yields were obtained from plots showing approximately 13 per cent. of moisture. There is some evidence, although it is not conclusive, to show that there is an interrelationship between the mean temperature during the stolon formation period and the moisture optimum. It is important to note that the peak of the stolon formation period is in March for the major plantings at Hastings and LaCrosse.

III. Effect of temperature on yield and grade of tubers

A comparison of the soil temperature with that of the air, the latter recorded by the United States Weather Bureau field station at Hastings, is shown in figure 4, covering the major portion of the potato growing

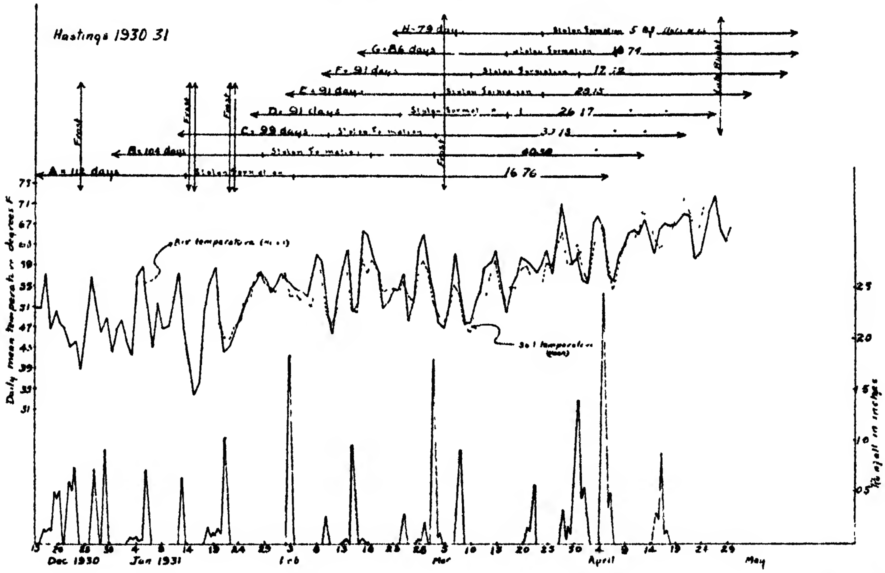

Fig. 4. Phenological relations prevailing during the potato growing season of 1930–1931 at Hastings, Florida. Number of days indicated for each planting represents time from planting to harvest. Note that frost occurred on six different days, damaging the plants of *A* severely during the stolon formation period, and those of *D* less seriously for the similar stage of growth, resulting in reduction in yield in both instances. The low yields for plantings *G* and *H* were primarily due to the short growing period subsequent to stolon formation, owing to blight incidence. Note that the soil temperature was lower during March and higher during April than corresponding temperature of the air.

season of 1930–31. The mean soil temperature for the period shown was 62.9° F., while the mean air temperature was 63.2° F. for the same period. It seems important to note that during March the mean soil temperature was nearly 3° below the air temperature, while at the end of the season during April the soil temperature rose above the air temperature, thus

making the two means for the period practically equal. Similar relations were found for the other years of this study.

In figures 5 and 6 are shown the average soil temperatures prevailing during the stolon formation period (28–50 days) for the successive plantings at LaCrosse during 1927–28 and for Hastings 1930–31, respectively, as they are related to yields and moisture, together with *Rhizoctonia* prevalence at LaCrosse. These examples are typical. The close coincidence of *Rhizoctonia* prevalence with changes in moisture is particularly interesting.

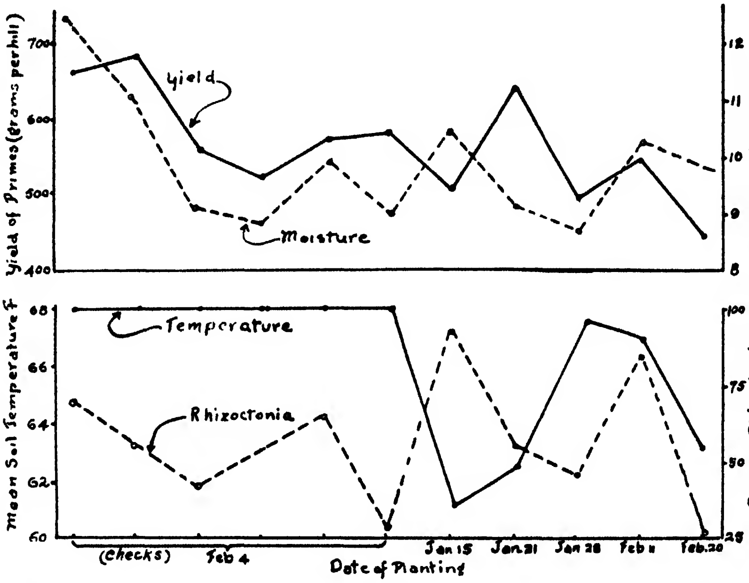

FIG. 5. When the temperature range is between 53° and 68° F. there seems to be little measurable effect upon potato yields. There is no apparent correlation between them. Both the moisture and the *Rhizoctonia* frequency curves, however, show direct correlation with yield. The one exception is noted with the January 15 planting where low temperature and high moisture favored the very high *Rhizoctonia* incidence (91 per cent.).

The experiments for the five years indicated that the mean temperature plays a relatively less important part in determining yields than does soil moisture. Only in two cases was the temperature so cold that germination and the initial growth were seriously retarded, namely, the January 15 planting of 1928 and the January 1 planting of 1931. There is some evidence in the correlation to show that the high temperatures during dry Aprils militate against high yields.

Temperature indirectly affects yields since diseases such as *Rhizoctonia* and Late Blight thrive best under relatively cool moist conditions, as shown

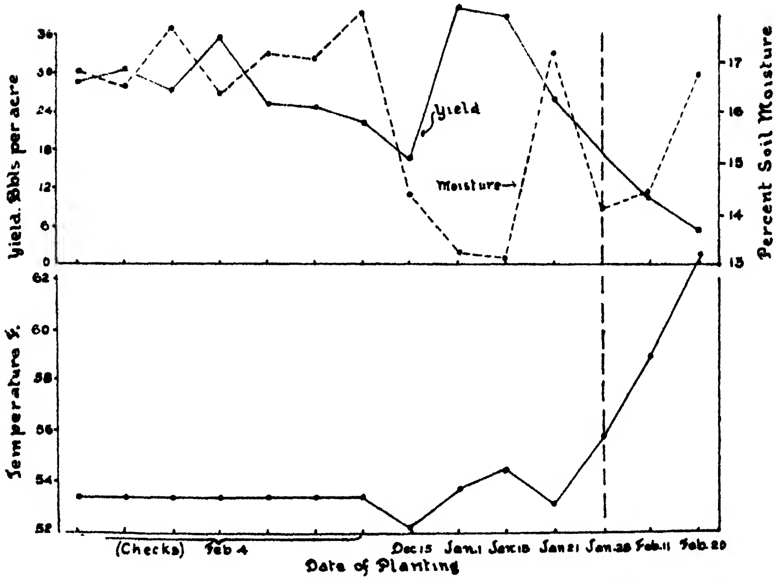

FIG. 6. Temperature effects are apparently of little significance during the period of stolon formation as judged by yield. Note that the checks (February 4 planting) were grown under the same temperature but under different moisture conditions and that the latter factor showed a decided inverse relationship to yield, with the possible exception of the December 15 and January 28 plantings. Mean temperature for the former period was unusually low, retarding growth visibly. Plantings from January 28 to February 20 were prematurely diseased by Late Blight.

by RICHARDS (6) and by our own experiments. The effects of freezing temperatures upon the growth and yield of potatoes are given in a separate publication. The chief conclusion was that the period of stolon formation (28th–50th day after planting) is a critical one, and that when vines are frozen to the ground at that stage the yields are reduced more than if they are frozen at any other time. It is important, therefore, to know the probabilities of the occurrence of freezing weather at various stages of potato growth in the Hastings and LaCrosse areas. Based upon Weather Bureau records of 40 years, the data shown in figures 7 and 8 are at least highly suggestive. The coldest weather usually comes during the first two decades of January and in the same intervals during February, as shown in figure 8. The time of stolon formation (critical period) occurs during the first half of February in plantings made prior to January 15–20. When the plantings are deferred to the last ten days of January, however, then the critical period falls during the milder weather which characterizes the latter part of February and the first days of March (fig. 7). This may be one reason why the late January plantings, over a long period of years, are said by growers to have yielded the largest crop of marketable tubers.

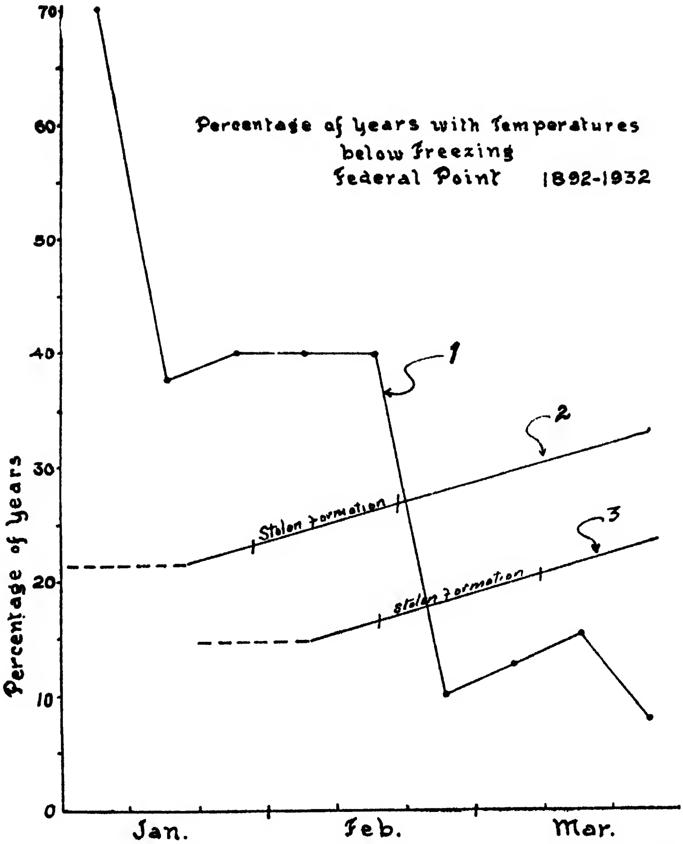

FIG. 7. Curve 1 shows freezing frequencies at 10-day intervals expressed in percentage of years during the months of January, February, and March. Two growth curves for potatoes are superimposed. Curve 2 represents the distribution of growth stages for plantings made about January 1. Curve 3 shows the distribution for plantings made about January 20. Dotted portion of curves 2 and 3 represents the time prior to appearance of sprouts above-ground. Note how stage II of curve 2 coincides with the high frequencies of cold weather the first two decades of February, while the same stage in curve 3 coincides with the milder weather the last of February and the first part of March.

When the mean monthly temperatures prevailing during January, February, March, and April, respectively, are compared with the yield of no. 1's over a 10-year period, some interesting relations are shown.[7] Cool Januaries and warm Marches seem to favor the growth and yield of no. 1 tubers (fig. 1). There is also an indication that cooler than normal

[7] The correlation coefficients for yields and mean temperature for the months of January, February, March, and April are -0.51 ± 0.15, 0.20 ± 0.20, 0.51 ± 0.16, and -0.20 ± 0.20 respectively.

weather in April increases yield. These facts suggest that our temperature curve in Florida is upside down as it relates to yields. As a matter of fact, it is almost precisely inverted as compared with the normal temperature curve for Maine as shown in figure 9. The average temperature

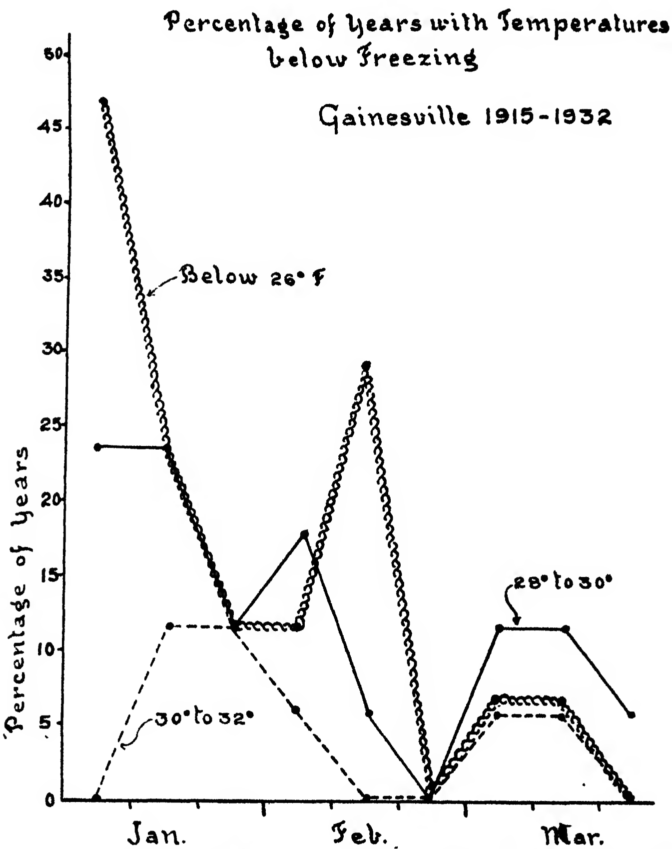

FIG. 8. Freezing frequencies for January, February, and March at 10-day intervals expressed in percentage of years. Subfreezing temperatures have been divided into three groups: 32°–30°, 28°–30°, and 26° and below. Note the high frequencies of the colder temperatures during the first part of January and the second 10-day period in February. The most frequent freezing temperatures for March are between 28° and 30°.

range during the last two months in Maine should be ideal for starch storage according to BUSHNELL's findings (1), while in Florida the rate of respiration is too high.

Discussion

The data show potatoes to be very sensitive to soil moisture variation, especially during the period of stolon formation. These findings accord

well with those of WIDTSOE (8, 9, 10), HARRIS (4, 5), and SMITH (7). In an area like Hastings, where irrigation water is easily available, there should be no reason why potatoes need suffer for water during this period. In fact, the most usual problem would be to prevent too much soil moisture. This of course suggests the need for better drainage. The opposite conditions prevail at LaCrosse where somewhat different soil and subsoil conditions obtain and where better drainage is facilitated by the greater slope of the land.

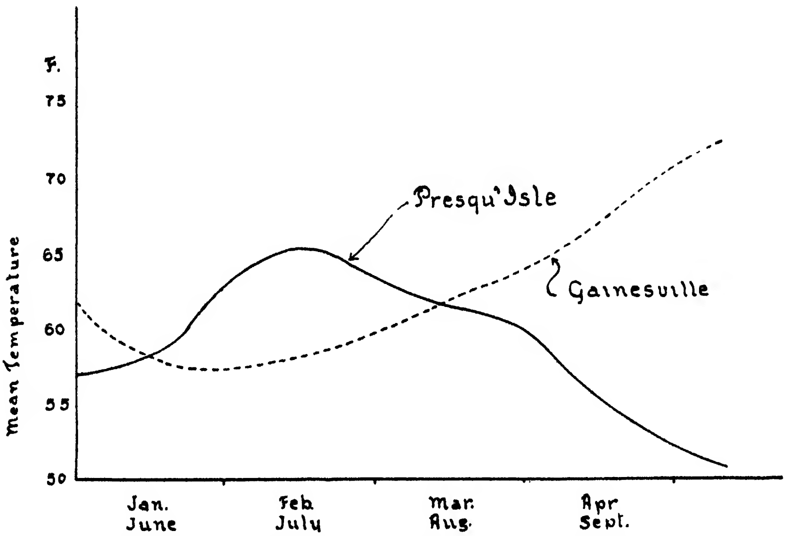

FIG. 9. Smoothed mean daily temperature data covering ten years (1917–1926 inclusive) for Presque Isle, Maine, and Gainesville, Florida, during their respective potato growing seasons. Note almost precise inversion of temperature curves. Correlations between yield and temperature at Hastings indicate that January and April are too warm while March is too cold.

There is an interesting question regarding the possible recurrence of high rainfall during March (period of stolon formation). If there are cycles, when do they come? In order to answer this question the rainfall record of March at Jacksonville since 1872 was submitted for harmonic analysis.[8] Among the cycles disclosed there were two of outstanding importance, approximately 10 and 7.5 years respectively. The reality and importance of these may be judged by the fact that the series of wet years in these cycles yielded an average of 199 and 223 per cent. more rainfall, respectively, than the corresponding series of dry Marches. It should be remembered that dry Marches were found to foster larger yields at Hastings.

[8] The analysis was made through the courtesy of DAYTON C. MILLER, Case School of Applied Science, Cleveland, Ohio.

The potato is apparently not so sensitive to the temperature variations that normally obtain in the Hastings area,—54° to 65° F. When mean monthly temperatures above or below these limits occur there appears a noticeable limitation of yield. The inversion of the temperature curve in Florida as compared with that in Maine may explain in part the lower yields that are common to Florida.

Summary

1. There is a considerable latitude admissible in the time of planting Irish potatoes in the LaCrosse and Hastings areas, ranging from mid-December to the middle of February. The two-week period extending from the middle of January to the first of February gave the largest average yields.

2. The period of stolon formation, extending from about the 28th to the 50th day after planting, appears to be a critical one in the development of the potato as measured by yield of primes. The peak of this critical period is reached about the 35th to the 40th day after planting.

3. The soil moisture content during the critical period seems to be the chief factor involved in determining yields, the optimum soil moisture content being about 12 to 13 per cent., based upon oven-dry weights.

4. In the LaCrosse areas the soil moisture rarely reaches the optimum and therefore too little moisture is the limiting factor in production. On the other hand, the soil moisture at Hastings is usually well above the optimum owing to a more impervious subsoil, so that too much moisture is the limiting factor in production there. Better drainage at Hastings and a need for irrigation at LaCrosse are indicated.

5. The month of March seems to be a critical one for the average planting, since it approximates the time of stolon formation. For Hastings a warm, relatively dry period extending from late February through March is favorable to large yields, and *vice versa.*

6. Temperature effects seem to be of lesser importance in their influence upon yields, although there is evidence to show that the temperatures prevailing during January and April are too high while March temperatures are too low for maximum yields.

7. March temperatures tend to be below normal when the precipitation is above normal and both of these factors are inimical to large yields.

8. There are four periods or cycles in March rainfall of approximately 30, 15, 10, and 7.5 years respectively. The latter two have recurred over the past 60 years so that the wet Marches show 199 and 233 per cent. increase of rainfall, respectively, over the corresponding dry years.

9. When potatoes are planted during the latter half of January the critical period of stolon formation follows the period of greatest freezing

frequency, and therefore reduces the chance of low yields due to freezing injury.

The writer is indebted to Dr. DAYTON C. MILLER of the Case School of Applied Science, Cleveland, Ohio, for the analysis of the rainfall data and to Mr. J. W. KITE of LaCrosse and Mrs. GEORGE V. LEONARD of Hastings for the courteous and able assistance given in the cultural practices and for the use of the land upon which the tests were conducted.

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CHANGES IN NITROGEN COMPOUNDS IN THE WHEAT GRAIN AT DIFFERENT STAGES OF DEVELOPMENT[1]

GEORGE L. TELLER

(WITH TWO FIGURES)

Introduction

Marketable wheat is mostly considered as having reached a full stage of maturity. There is, however, much wheat on the market whose development has been checked at various stages short of full maturity. This is a somewhat variable condition, depending upon climate, season, soil, moisture, and time of harvesting as well as upon the type of grain. Much of this immature wheat has valuable, and under some conditions superior, milling qualities. The results of a systematic study of changes which take place during growth and development of the grain are therefore of practical value as well as of scientific interest.

ALSBERG, in considering the character of the proteins laid down during development, states (**1**, p. 236), "With the exception of WOODMAN and ENGLEDOW (**13**) almost no one has distinguished between the two proteins of which gluten is composed, glutenin and gliadin." ALSBERG draws this conclusion from the reading of BAILEY (**2**) and refers his readers to BAILEY for a review of the literature. BAILEY'S notes on this subject are not complete, although he gives the results of WOODMAN and ENGLEDOW in much detail. Their report, and the methods they used, show much to be desired. Among other things the recording of ammonia nitrogen should be explained. Even in the mature grain it is reported as high as 0.10 per cent. and higher. It is also clear that a large part of the gliadin was carried away in the 1 per cent. salt solution which they used before extraction with alcohol was begun. The amounts of gliadin shown, therefore, are much too low.

Methods

The writer made an extended study of wheat and its products during the years 1893 to 1898 inclusive. In 1893 researches of OSBORNE and VOORHEES (**7**) on the proteins of wheat were then new. Based upon their findings, convenient methods for making systematic series of determinations on the amounts of different nitrogen compounds in wheat and its products were desired. It was a pioneer undertaking and the nature of the substances to be separated does not tend toward exactness. After a careful preliminary study certain corrections for overlapping solubilities were

[1] Contribution from The Columbus Laboratories, Chicago, Illinois and the Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

found to be necessary. With these corrections the determinations on different wheats and products were found to be wholly consistent, and have been so found in numerous instances since that time.

These methods were first published in 1896 (**8**), and with some modifications again in 1898 (**9**). The original methods may be found in LEACH (**6**) and earlier editions where they were introduced by OSBORNE. They were used in studying the proteins of wheat bran (**11**) and are given in detail in the paper recording the results of that study.

Briefly, gliadin and the non-protein nitrogen compounds of wheat are soluble in neutral alcohol (sp. gr. 0.90). Little if any of the albumin and globulin are soluble in it. A 1 per cent. sodium chloride solution dissolves both of these, all the non-protein compounds, and some gliadin. Under uniform conditions the considerable amount of gliadin dissolved is very uniform (**8**). The protein compounds are completely precipitated from the salt solution by phosphotungstic acid, and the non-protein nitrogen in the filtrate can be readily determined. This must be deducted from the alcohol-soluble nitrogen to give the gliadin. The albumin and globulin may be precipitated from the salt solution by alcohol and their nitrogen determined in the precipitate as collected, or they may be found by difference when deductions for dissolved gliadin nitrogen and the non-protein nitrogen are made. The glutenin nitrogen is found as the difference between the total nitrogen and the sum of the nitrogen of the other compounds. Gliadin and glutenin are the two proteins which together form the greater part of gluten as it is ordinarily separated from flour by hand washing. All nitrogen determinations were made by the Kjeldahl method or the Gunning modification. The factor 5.7 was used for calculating protein from the nitrogen.

Experimentation

During the years 1897 and 1898, wheat at Fayetteville, Arkansas, was cut daily during the development of the grain and for several days after full ripeness. A portion of each cutting was put to dry on the straw in a well ventilated barn loft on a wire netting suspended from the roof to protect from rodents. From another portion the heads were cut and dried in like manner. In due time each lot was threshed by a threshing machine cylinder supported in a suitable wooden frame with box attachment to receive the threshed grain. This was cleaned in a farm fanning mill and stored in jars for analysis. During the first year a part of that dried on the straw was milled in a small roller mill to obtain the pure endosperm or fine flour for the analysis. This was carefully separated from the remainder by a series of hand sieves with bolting silks.

An extended analysis was made on the successive lots of wheat to determine total mineral matter, fat, different forms of carbohydrates, and

different nitrogen compounds. This paper for the most part deals only with the last. The methods used were those just described and the determinations included total nitrogen, non-protein nitrogen, the albumin and globulin (edestin and leucosin), the prolamine (gliadin), and the glutelin (glutenin). The number of kernels in 10 gm. of that ripened on the straw was also determined. The latter is a helpful guide to the condition as to development. The percentages of starch shown in table X contribute also to that end.

For convenient comparison of data the results of analyses of the several daily cuttings are grouped as the average of three days each. However, the wheat from each day was analyzed separately for the nitrogen and its compounds, both that dried on the straw and that in the head cut from the straw. For other determinations the three-day samples were mixed before analyses and the analyses were made only on those dried on the straw.

Detailed results of these several analyses of the grain of the harvest of 1897 have already appeared (9). Results of the protein separations for 1898 have not heretofore been published, although the more general analyses were published in a paper discussing carbohydrates (10). Results of both years are given here as either without the other would lose some-

TABLE I

APPROXIMATE STAGE OF DEVELOPMENT OF WHEAT WHEN CUT AS RECORDED FOR PERIODS OF THREE DAYS EACH. SERIES OF 1897

PERIOD	STAGE OF DEVELOPMENT
I	A little past blossom; grain set
II	Berries one-half to full length of ripe grain
III	Crushed berries exude a thin milky liquid; lower leaves beginning to die
IV	Grain well in milk
V	Heads and kernels well developed; interior of grain a thin dough
VI	Grain in dough
VII	Grain in stiff dough; straw becoming yellow at butt; grain shells a little with rough handling
VIII	Straw in field much yellowed but still decidedly green
IX	Grain oozes a thin liquid when crushed between the thumb nails; contents still slightly viscid; straw still a little green
X	Wheat fit to cut at beginning of this period; straw has lost all its green color and is dark purple immediately below the heads; berry nearly dry and may be crushed between the thumb nails but without contents adhering to them
XI	More than ripe; straw bright and stands up well
XII	More than ripe; straw bright and stands up well
XIII	More than ripe; straw bright and stands up well
XIV	More than ripe; straw bright and stands up well

thing of its value. The comparisons as shown in the charts (figs. 1, 2) are especially instructive.

TABLE II

NUMBER OF KERNELS IN 10 GM. OF WHEAT RIPENED ON STRAW

Period	Series of 1897	Series of 1898
I	3125	9632
II	1964	4628
III	1080	1708
IV	746	834
V	494	519
VI	412	426
VII	333	362
VIII	296	321
IX	269	298
X	254	293
XI	253	293
XII	252	285
XIII	247	286
XIV	248	. .

TABLE III

AVERAGE TOTAL NITROGEN IN PERCENTAGE DRY MATTER IN WHEAT GRAIN DRIED ON THE STRAW AND IN HEADS CUT FROM THE STRAW. SERIES OF 1897

Period	Head series	Straw series	Difference
	%	%	%
I	3.85	3.68	0.17
II	3.63	3.25	0.38
III	3.20	2.85	0.35
IV	2.90	2.63	0.27
V	2.73	2.55	0.18
VI	2.45	2.36	0.09
VII	2.64	2.45	0.19
VIII	2.73	2.69	0.04
IX	2.75	2.78	−0.03
X	2.95	2.95	0.00
XI*	3.01	2.91	0.10
XII	3.11	3.02	0.09

* Average two days only.

TABLE IV

AVERAGE TOTAL NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL DRY MATTER OF WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN				
	TOTAL	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%	%
I	3.68	1.04	1.63	0.44	0.57
II	3.25	1.13	1.40	0.44	0.28
III	2.85	1.14	1.08	0.43	0.20
IV	2.63	1.10	0.95	0.40	0.18
V	2.55	1.10	0.91	0.38	0.16
VI	2.36	1.08	0.84	0.33	0.11
VII	2.45	1.19	0.88	0.28	0.10
VIII	2.69	1.35	0.96	0.28	0.10
IX	2.78	1.49	0.91	0.29	0.09
X	2.95	1.58	0.93	0.34	0.10
XI	2.91	1.54	0.94	0.34	0.09
XII	3.02	1.62	0.94	0.35	0.11
XIII	3.03	1.59	0.98	0.34	0.12
XIV	3.17	1.68	1.01	0.36	0.12

TABLE V

AVERAGE NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL NITROGEN IN WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN			
	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%
I	28.4	44.2	11.9	15.5
II	34.7	43.1	13.7	8.5
III	39.9	37.8	15.1	7.2
IV	41.9	36.2	15.2	6.7
V	43.3	35.7	14.7	6.3
VI	45.6	35.6	13.9	4.9
VII	48.5	35.8	11.4	4.3
VIII	50.0	35.9	10.3	3.8
IX	53.5	32.7	10.4	3.4
X	53.3	31.5	11.8	3.4
XI*	52.9	32.4	11.5	3.2
XII	53.5	31.1	11.5	3.9
XIII	52.4	32.3	11.1	4.2
XIV	53.0	31.9	11.3	3.8

* Average two days only.

TABLE VI

AVERAGE TOTAL NITROGEN AND NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL DRY MATTER IN WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1898

Period	Nitrogen				
	Total	Gliadin	Glutenin	Edestin and Leucosin	Non-protein
	%	%	%	%	%
I	4.45	0.43	1.41	0.28	2.33
II	3.84	0.93	1.70	0.45	0.76
III	3.17	1.18	1.23	0.50	0.26
IV	2.67	1.09	0.97	0.44	0.17
V	2.56	1.11	0.90	0.41	0.14
VI	2.60	1.23	0.86	0.39	0.12
VII	2.66	1.29	0.86	0.39	0.12
VIII	2.65	1.31	0.87	0.37	0.10
IX	2.61	1.31	0.82	0.38	0.10
X	2.62	1.31	0.83	0.38	0.10
XI	2.61	1.33	0.81	0.37	0.10
XII	2.58	1.30	0.82	0.37	0.09
XIII	2.59	1.30	0.84	0.36	0.09

TABLE VII

AVERAGE NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL NITROGEN IN WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1898.

Period	Nitrogen			
	Gliadin	Glutenin	Edestin and Leucosin	Non-protein
	%	%	%	%
I	9.6	31.7	6.3	52.4
II	24.2	44.3	11.7	19.8
III	37.2	38.8	15.8	8.2
IV	40.8	36.3	16.5	6.4
V	43.3	35.2	16.0	5.5
VI	47.3	33.1	15.0	4.6
VII	48.5	32.3	14.7	4.5
VIII	49.4	32.9	13.9	3.8
IX	50.2	31.4	14.6	3.8
X	50.0	31.7	14.5	3.8
XI	51.0	31.0	14.2	3.8
XII	50.4	31.8	14.3	3.5
XIII	50.2	32.4	13.9	3.5

TABLE VIII

AVERAGE TOTAL NITROGEN AND NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL DRY MATTER OF ENDOSPERM OF WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN				
	TOTAL	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%	%
VI	2.03	1.19	0.62	0.19	0.02
VII	2.08	1.20	0.68	0.18	0.02
VIII	2.18	1.31	0.67	0.18	0.02
IX	2.21	1.33	0.68	0.18	0.02
X	2.31	1.41	0.70	0.18	0.02

TABLE IX

AVERAGE NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL NITROGEN IN ENDOSPERM OF WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN			
	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%
VI	58.6	30.8	9.6	1.0
VII	57.8	32.7	8.2	1.0
VIII	60.1	30.8	8.2	0.9
IX	60.2	30.8	8.1	0.9
X	61.0	30.3	7.8	0.9

Discussion

As shown in table II, there were more than three times as many kernels in the same weight of grain in the first period of the second year as in the same period of the first year. This striking difference is apparently due to at least four days' earlier cutting in the stage of growth. This is also apparent in the results of the analyses, especially as to the much higher nitrogen content and the much greater proportion of non-protein nitrogen in the first period of the second year's cutting.

Comparing the total nitrogen in the grain ripened on the straw and that in the heads cut from the straw (table III), we find a material decrease in the nitrogen in the former. This is apparently to be attributed to the transfer of carbohydrate substances from the straw into the grain. In

TABLE X

TOTAL NITROGEN AND STARCH* IN PERCENTAGE TOTAL DRY MATTER OF WHEAT CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH DURING TWO HARVEST YEARS. GRAIN DRIED ON THE STRAW

Series of 1897			Series of 1898		
Period	Total nitrogen	Starch	Period	Total nitrogen	Starch
	%	%		%	%
I	3.68	41.5	I	4.45	16.7
II	3.25	47.7	II	3.84	29.7
III	2.85	54.7	III	3.17	47.2
IV	2.63	59.4	IV	3.67	56.4
V	2.55	63.8	V	2.56	60.1
VI	2.36	65.3	VI	2.60	61.8
VII	2.45	65.7	VII	2.66	62.1
VIII	2.69	65.5	VIII	2.65	62.1
IX	2.78	65.4	IX	2.61	62.9
X	2.95	64.3	X	2.62	63.4
XI	2.91	65.2	XI	2.61	64.5
XII	3.02	64.4	XII	2.58	63.5
XIII	3.03	63.8	XIII	2.59	63.1
XIV	3.17	63.2			

* The starch as here shown corresponds closely to the amount found by diastase followed by acid hydrolysis but includes also a small amount of undetermined material, especially in the early periods.

the second year's cutting there was not such a marked and constant difference and the results are not here shown. The relations of the different forms of nitrogen compounds in percentage of total nitrogen in the straw series and in the head series were much the same during each year.

There was a steady decrease in percentage of nitrogen in the grain during the early period of both years (tables IV and VI). Following this there was during the first year a steady and marked increase up to and including the final cutting. During the second year this increase was hardly more than begun when a decrease set in and continued to the end. Since these cuttings were made in nearly the same location and on the same variety of wheat (Fulcaster), it is clearly a seasonal difference. This decrease in nitrogen in the grain was found by KEDZIE (4, 5) in two years' cuttings in Michigan (1881 and 1893). An increase in nitrogen similar to that of the first year was found by BRENCHLEY and HALL (3) at Rothamsted, England, and by THATCHER (12) on American spring wheat. This variability is significant in connection with preliminary surveys of the nitrogen content of the coming wheat crop as now sometimes made from early sampling in the field.

The material increase of nitrogen in the endosperm or flour portion of the grain over the periods during which this material was separated is of even greater interest (table VIII). The percentage of total nitrogen in the dry matter is more than one-eighth greater in period V than in period VI.

In separating the endosperm an effort was made to secure only the purest of it. In doing this a large proportion of good flour material was rejected. That portion of the flour rejected lies closer to the bran and contains a distinctly larger proportion of nitrogen than that near the center of the kernel. Because of this the difference between the percentage of nitrogen in the endosperm as examined and in the whole wheat is greater than it would have been if all the endosperm had been included. When we compare the amount of gliadin in the endosperm in percentage of the total nitrogen, however, we find it greater than in the wheat. This is fully in accord with my previous findings made in 1896 and in 1932, which show a markedly higher proportion of gliadin in the endosperm than in the bran. The steady increase of gliadin in the wheat during the early stages of both years (tables V and VII; figs. 1, 2) is due in large measure to the increased filling of the grain with endosperm. It parallels somewhat closely the increase in the proportion of starch as shown in table X. That

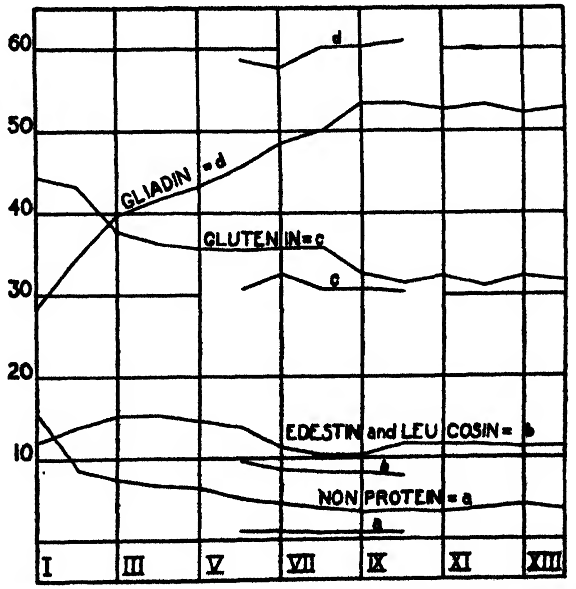

FIG. 1. Changes in proportions of nitrogen compounds in grain of wheat from early formation to past maturity; series of 1897. Short lines with letter indicate corresponding compounds in endosperm; figures show percentage; Roman numerals indicate period.

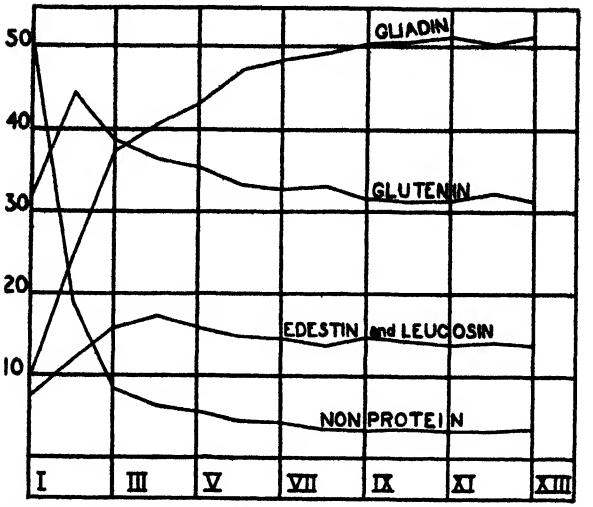

FIG. 2. Changes in proportions of nitrogen compounds in grain of wheat from early formation to past maturity; series of 1898. Figures show percentage; Roman numerals indicate period.

it is not wholly due to this cause is apparent from tables VIII, IX, and others.

The composition of growing plants gathered from the soil at different times is subject to so many causes for variation that it is extremely difficult if not impossible to adhere even fairly closely to the well established requirements of successful experimental work, namely, to allow variations along one line only. Nevertheless, when correctly interpreted, the combined results of repeated attempts often lead to findings of substantial value. At all events they are in accord with nature and the industries that make use of nature's products.

Summary

1. Among results of two years' careful study of the development of wheat grain, as shown by the analyses of daily cuttings from its first formation to far past maturity, it was found that the large proportion of non-protein nitrogen compounds which are predominant at the very earliest formation of the kernel are quickly changed into the different forms of protein characteristic of the wheat grain. Over the earlier periods there was a continuous increase of gliadin and a corresponding but less marked decrease in the proportion of glutenin. In these earlier periods there was also a slight falling off in the proportion of the combined non-gluten proteins, the albumin and globulin. These changes are in part due to the filling of the grain with endosperm. This contains a much larger proportion of gliadin and a smaller proportion of glutenin and the non-gluten nitrogen compounds than the bran.

2. For both years there was a decrease of total nitrogen in the grain over the early periods, which is also in accord with the results of increased endosperm. Owing to seasonal differences there was for one year a steady increase in the proportion of total nitrogen in the grain throughout the later periods. For the other year there was a decrease in this proportion of nitrogen. Both of these conditions as to the relative amounts of total nitrogen in the grain are in conformity with findings of certain other investigators who have pursued that feature of the study.

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DISTRIBUTION OF ROOTS IN POROUS AND NONPOROUS PLANT CONTAINERS[1]

LINUS H. JONES AND HENRI D. HASKINS

(WITH THREE FIGURES)

Introduction

Many years of experience in growing plants in clay pots have taught the horticulturist to judge the needs of his plants by the condition of the root system. The so-called pot-bound condition, in which the root system appears as a mass of interlacing roots between the pot and the soil mass, is a criterion that is applicable only to this type of container, *i.e.*, the porous clay pot.

JONES (**1**) has shown that plants may be grown in nonporous containers and that such plants are equal to those grown in the conventional clay flower pot. The study of root systems in both porous and nonporous plant containers shows a striking difference. In the porous pot, the root system is almost wholly outside the soil mass and the core of soil within may be easily removed. In the nonporous pot, there are relatively few roots on the outside of the soil mass and it is exceedingly difficult to remove the soil from the roots, which ramify throughout the soil. This condition was observed by WHITNEY and CAMERON (7) in 1904 when experimenting with soils in wire baskets covered with paraffin. Such plant containers are nonporous. These investigators state: "In no case has there been found any evidence of any effort on the part of roots to develop toward the sides of the pot. On the contrary, they grow freely throughout the soil." The wire basket covered with paraffin was the outcome of failures to keep the root system within the soil, for in glass tumblers this particular soil contracted, leaving an air space saturated with water vapor between the soil and the wall of the tumbler. These investigators further noted that they obtained an even distribution of soil moisture within these paraffined wire baskets.

Top-root ratios in porous and nonporous plant containers

A comparison of the root systems of plants grown in porous and in nonporous pots invariably showed a larger root system in the porous pot; moreover, it was observed that the soil in nonporous pots always contained a greater amount of moisture than did the soil in porous pots.

In order to obtain more definite information on top-root ratios of plants grown in porous and nonporous containers, the following experiment was carried out.

[1] Contribution no. 186 of the Massachusetts Agricultural Experiment Station.

Sixty 3-inch clay pots and sixty glass tumblers, each holding the same volume of soil as a 3-inch clay pot, were planted with tomato seedlings. The containers were watered each day and checked every alternate day by weighing in order to maintain a fairly even moisture content of the soil. The soil in the clay pots, however, frequently became dry owing to excessive evaporation from the walls of the pots. It was necessary to apply twice as much water to the clay pots as was applied to the tumblers. At the end of 33 days, the plants in the clay pots were showing signs of becoming pot-bound, *i.e.*, the roots were heavily massed against the wall of the pot and even projected above the surface of the soil at the region of contact of soil and pot. At this time the soil was carefully washed from the roots, and the top of each plant was cut off just above the first root. The tops and roots were dried in an oven at 101° C. and weighed. Table I gives the average weights obtained.

TABLE I

AVERAGE DRY WEIGHTS OF 60 TOMATO PLANTS GROWN IN CLAY POTS AND OF 60 TOMATO PLANTS GROWN IN GLASS POTS FOR 33 DAYS

TYPE OF POT	AVERAGE WEIGHT OF ROOTS	AVERAGE WEIGHT OF TOPS	AVERAGE TOTAL WEIGHT OF PLANT	AVERAGE T/R RATIO
	gm.	*gm.*	*gm.*	
Clay	0.3853	1.2750	1.6603	3.31
Glass	0.3429	1.3773	1.7202	4.02

Although an attempt was made to maintain a fairly uniform moisture content in the soil, observations indicated that only by frequent weighings over the daily period could the moisture in the soil of the clay pots approximate the uniform moisture content of the glass tumblers. The result of this experiment, as shown by table I, demonstrates that a somewhat smaller root system in the glass container produced fully as large a plant as was obtained in the clay pot. However, the difference was slight in the total weights of plants (roots and tops) grown in both types of containers, although as a matter of fact the average weight of the whole plant grown in glass was somewhat greater than that obtained in clay. As noted in previous experiments, the root systems were distributed according to the type of container in which they were growing, the roots in the clay pot being for the most part massed on the outside of the soil and those in the glass tumbler being well distributed throughout the soil mass. Figure 1 illustrates these two types of root distribution and figure 2 shows the two root systems with soil removed.

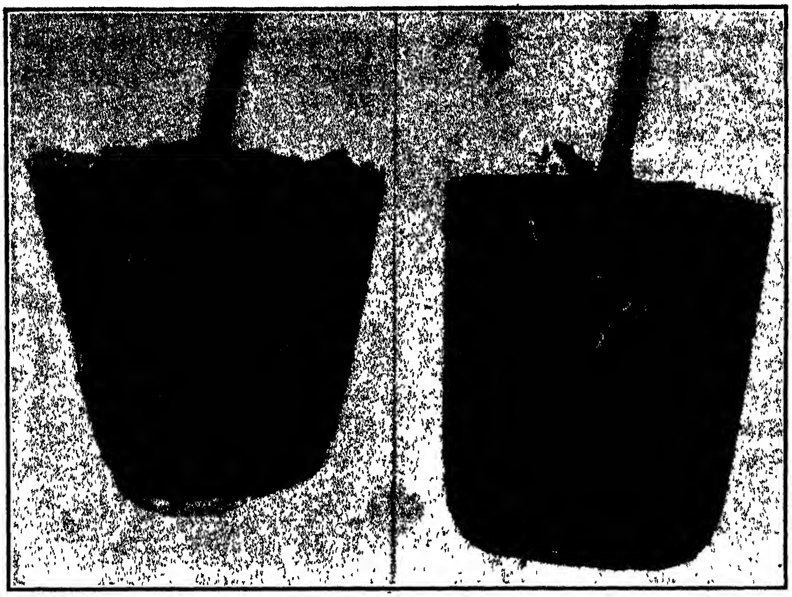

FIG. 1. Distribution of roots in porous pot (left) and nonporous pot (right). Tomato plants 40 days old.

Relation of nutrients to root distribution

It is generally believed that roots grow between the wall of a clay pot and the soil mass in order to obtain the air that is supposed to pass through the wall of this type of plant container. In fact, it is the presence of the root system at this point of contact between pot and soil that is offered as proof that air does pass through such walls. The demonstration by JONES (2) that a rubber vacuum disc will remain fixed on the moist wall of a clay pot for over 30 days disproves the popular belief that a clay flower pot is porous to air. It is necessary, therefore, to find some other explanation for the massing of roots near the wall of the clay pot.

There is considerable evidence in the literature to show that under normal moisture conditions root formation takes place where there is an available source of nutrients, and, up to a certain point, the amount of root formation increases directly as the nutrient supply increases. LIVINGSTON (4) found an increased branching of roots as the amount of manure was increased in a poor soil. The tremendous development and branching of roots within drain tiles, even in regions where there is plenty of soil moisture, is a response to a nutrient factor. It has been noted that an unusually great number of roots will develop in manure layers where manure is used alternately with soil, or where a layer of clay has absorbed some of the nutrients that have leached through sand.

FIG. 2. Root systems of figure 1: left, porous pot; right, nonporous pot.

The porous pot not only absorbs moisture, but it also provides a means of moving soil moisture into the air through the wall of the pot. Such pots are capable of losing twice as much water through the wall of the pot as from the surface of the soil. A theory that the distribution of roots in plant containers is tied up directly with plant nutrients and is indirectly related to soil moisture movements is partly supported by the following observations and chemical analyses.

The work of KNOTT and JEFFRIES (3) indicates the large amount of nitrogen that is absorbed by the clay pot from the soil within. In the opinion of the writers, roots develop at the point where the relatively large amount of available nitrogen is concentrated by the movement of the water drawn through the wall of the pot by the force of evaporation. The successful horticultural practice of using small pots for small plants may be explained on the basis that in the small pot the roots can more quickly reach this source of nutrients. A small plant in a large pot grows slowly

compared with a small plant in a small pot. The practice of placing poorly rooted cuttings nearer the wall of the pot than the center in order to encourage better growth may be interpreted to mean that the roots are there much nearer the region where nitrogen and other nutrients will be concentrated. Tests have confirmed these practices with the clay pots; but when nonporous pots of paper or glass were employed, the size of the pot or the position of the seedling or cutting had no effect on subsequent growth, and this growth was equal to any growth obtained in the clay pots.

It appears that the soluble salts in the soil, which include the nutrients, move in the same direction as the moisture. The marked lateral movement of moisture that occurs in a clay pot is entirely absent in a nonporous pot. Although vertical movement of moisture occurs in both the clay and nonporous pots, any upward movement of salts is counteracted by top watering which leaches these salts downward. The more even distribution of soil moisture in the nonporous pots compared with moisture distribution in clay pots probably has associated with it a more even distribution of nutrients, which may account for the ramifying root systems in the nonporous plant containers.

A demonstration that there is an unequal distribution of nutrients in clay flower pots is shown in the following results, which are taken from some preliminary experimental work that bears upon the subject of lateral movement of nutrients in the soil of a clay flower pot. To each of six 6-inch flower pots containing soil were added 7.5 gm. of a 5–8–7 fertilizer, practically all of which was in a soluble form. The soil was kept fallow and the pots watered each day. The pots were kept on a bed of moist cinders. At intervals of 4, 8, and 12 weeks, two pots were sampled in four cores according to the following diagram (fig. 3). Portions of each sample were washed with like amounts of distilled water, the solutions evaporated, and a determination made of the total water-soluble solids. It was found that the greatest concentration of water-soluble solids was always obtained in the center core (*A*), and that in general there was a definite reduction of total water-soluble solids progressing through cores *B*, *C*, and *D*. Each of these cores

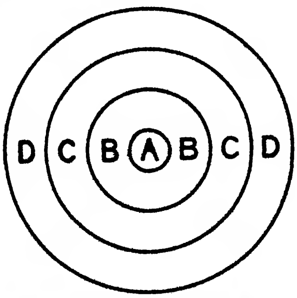

FIG. 3. Diagram showing relative positions of cores *A*, *B*, *C*, and *D*.

contained less total water-soluble solids as the sample taken became closer to the wall of the pot. In table II are given the results obtained from the 4-, 8-, and 12-week samples.

TABLE II

TOTAL WATER-SOLUBLE SOLIDS IN THE SEVERAL CORES DRAWN FROM 6-INCH POROUS CLAY POTS. RESULTS CALCULATED TO A DRY MATTER BASIS

	Total water-soluble solids			
	Core A	Core B	Core C	Core D
	%	%	%	%
4-week samples	0.8306	0.6144	0.5229	0.3899
8-week samples	0.9368	0.5878	0.5646	0.3941
12-week samples	0.7710	0.4650	0.5816	0.4364

The detailed analysis of a composite of the total water-soluble solids from the several cores, drawn at the three intervals, is given in table III.

TABLE III

DETAILED ANALYSIS OF COMPOSITE SAMPLES OF CORES DRAWN AFTER 4-, 8-, AND 12-WEEK INTERVALS. RESULTS COMPUTED TO A DRY BASIS OF THE WATER-SOLUBLE SOLIDS

	Core A	Core B	Core C	Core D
	%	%	%	%
Total nitrogen	0.0113	0.0076	0.0101	0.0046
Nitrogen as nitrates	0.0082	0.0045	0.0067	0.0018
Nitrogen other than nitrates	0.0031	0.0031	0.0034	0.0028
Potash	0.1022	0.0704	0.0832	0.0554
Phosphoric acid	0.0054	0.0042	0.0028	0.0028
Calcium oxide	0.1627	0.0947	0.0765	0.0392
Magnesium oxide	0.0390	0.0257	0.0242	0.0189
Chlorine	0.0282	0.0203	0.0255	0.0216
Soluble sulphates (SO_3)	0.2743	0.1638	0.1410	0.0689
Total of soluble constituents as analyzed	0.6231	0.3867	0.3633	0.2114
Residue of total soluble solids not recovered by analysis*	0.2230	0.1690	0.1931	0.1954
Percentage of total soluble solids recovered by analysis	73.64	69.59	65.29	51.97

* It seems probable that the residue of the total soluble solids not recovered by these analyses would be largely soluble organic matter, sodium compounds, and small amounts of such substances as Mn, Fe, Al, and SiO_2.

This table shows in the analysis of the total soluble solids, the sum of the seven determinations on composite sample *A* was 66 per cent. more than on

sample *D;* about 42 per cent. more than on sample *C;* and 38 per cent. more than on sample *B*. This shows a diminishing quantity of soluble soil constituents from the center core (*A*) to the outer soil region (*D*), and agrees with the data in table II which show the percentages of total water-soluble solids from each sample. The data in these two tables indicate that there is a pull of soil salines toward the pot wall, *i.e.*, lateral diffusion, and that the pot has absorbed a considerable quantity of the soluble nutrients. The fact that core *D* contains a smaller quantity and cores *C* and *B* a somewhat larger quantity of the water-soluble constituents might indicate that these compounds had been absorbed by the pot walls. Naturally, as the concentration of the soil solution was less in the outer layers of the soil, in the presence of a lateral movement of soil moisture induced by the heavy pull caused by evaporation through the pores of the pot the supply of soluble nutrients must naturally come from cores *B* and *A*, which function as reservoirs for both water and the soluble constituents which the water carries.

That the pot does absorb soluble plant food constituents is illustrated by the chemical analysis (given in table IV) of a gray, crystalline deposit scraped from the outer surface of clay pots which had seen several years of service in a greenhouse.

The literature that might bear directly on this subject suggests that a plant container with an evaporating wall may cause a lateral diffusion of soil salines. McHargue (5), working with manganese, discusses this point.

TABLE IV

Chemical analysis of crystalline deposit scraped from surface of clay pots

	%
Moisture in vacuum oven at 55° C. for 10 hours	2.30
Organic and volatile matter exclusive of free moisture	24.90
Total nitrogen	0.55
Ammoniacal nitrogen	None
Nitrate nitrogen	0.32
Water-soluble organic nitrogen	0.05
Water-insoluble organic nitrogen	0.18
Phosphoric acid	0.79
Iron and aluminum oxides	0.76
Calcium oxide	26.28
Magnesium oxide	1.08
Sulphates (SO_3)	35.09
Potassium oxide	0.39
Sodium oxide	0.89
Soluble silica	0.40
Silica insoluble in dilute hydrochloric acid (most likely a part of the clay pots)	7.20

He found that crocks that were not well glazed showed crystalline deposits of nutrients after the crocks had been in use a short time. The poor glazing permitted the migration of moisture which carried the mineral nutrients in solution through the walls, so that subsequent evaporation of the moisture caused deposition of the mineral nutrients on the outside. In a later publication McHargue (6) states that "the wall of earthenware jars which are in general use for pot experiments may be sufficiently porous to absorb mineral nutrients from soil or sand cultures, and these may become available and affect the growth of plants in subsequent experiments." Knott and Jeffries (3) found that new flower pots absorbed considerable nitrogen, the pot competing more successfully for this element than the plant. They also suggest that other soil salts may be similarly absorbed.

These ideas are well confirmed by the behavior of plants, particularly of their root systems, in the cement flower pot. The pore spaces in the wall of the cement pot are so large that only when the soil within is excessively moist is there a movement of capillary moisture through the wall of the pot. During the greater part of the time, therefore, the wall of the cement pot is dry and porous to air when supporting plant growth, and for this reason the root system ramifies through the soil and is not found between the soil and the pot. The cement pot, typifying these characteristics, supplies a basis for believing that root distribution is not dependent on nor greatly influenced by aeration through the wall of the pot. The ramifying root system found in both cement and nonporous containers is associated with a lack of lateral movement of soil moisture, which in turn influences a lack of lateral movement of soluble plant food.

In the porous clay pots, however, movement of capillary water through the wall of the pots and the presence of deposited salts left on the pots by the evaporated water result in massing of root systems between the soil and the pot. As reported by Knott and Jeffries (3), a new pot produces a stunted, yellow, inferior plant, as the wall of the pot absorbs a considerable portion of the soil nutrients. The moisture relations in a new and an old clay pot are the same, but the nutrient relations are different. It is reasoned from the evidence presented, therefore, that the presence of nutrients is the most important factor in root development, and that distribution of nutrients is related to soil moisture movements.

Summary

1. Root systems in porous pots are found, for the most part, outside the soil mass and next to the wall of the pot. In nonporous containers the root systems ramify throughout the soil with but a very small proportion next to the wall of the plant container.

2. The size of a root system is influenced by the moisture content of the soil. If the moisture content of the soil in a porous pot is maintained approximately equal to that in a nonporous container, the root systems will be nearly equal.

3. A determination of water-soluble solids made at intervals of 4, 8, and 12 weeks showed an unequal distribution of this material in the porous pots. In general, the amount of water-soluble solids decreased directly as the concentric cores were measured from the center to the periphery.

4. A detailed analysis of the composite samples of the four cores showed that all the elements for which determinations were made followed the same general rule of decreasing in concentration from the center outward.

5. The cement pot is porous to air, but does not maintain a lateral movement of moisture. The root system in a cement pot ramifies throughout the soil as does the root system in a nonporous pot.

6. The distribution of root systems in plant containers is associated with the distribution of nutrients, which in turn is affected by soil moisture movements.

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THERMAL CONDUCTIVITY OF STORED OATS WITH DIFFERENT MOISTURE CONTENT[1]

A. L. Bakke and H. Stiles[2]

(WITH ONE FIGURE)

Much has been written about the heating that takes place in damp or moist grain, but apparently no measurements have been made of thermal conductivity. In a previous publication by Bakke and Noecker (1), it was shown that in oats in storage there is considerable variation in oxygen consumption even with oats of low moisture content. There is a general tendency for respiration to increase with the moisture content, although there is considerable variation. In certain localized areas where the grain is not sufficiently dry for storage, spoilage takes place owing to rapid oxidation of the grain. In these areas or "heat pockets" the heat generated has been largely retained owing to the fact that the grain is a poor conductor of heat. The work reported in this paper is an attempt to measure the thermal conductivity of oats stored at different moisture contents.

Stiles's method of measuring thermal conductivity

The thermal conductivity of oats was measured by the apparatus developed by Stiles (2, 3) in obtaining the thermal conductivity of heat-insulating materials. Briefly the method is to place the material to be tested between the ice vessel and the hot water tank, being sure that the upper temperature is constant at 32° F. Heat passage through the material to be tested is then determined by timing the water-level drop in the measuring tube which depends on the rate of melting of the ice in the cylinders.

The tank is a copper cylinder 14½ inches high and 16 inches in diameter, holding approximately 100 pounds of water. The water is heated by a Bunsen burner. On account of the large heat capacity of the water, it has been found possible after a little practice to keep the temperature of the water constant to within one-half degree for hours. The water is kept in circulation by a stirrer which consists of two sets of vanes mounted on a short shaft placed in a brass cylinder 3 inches in diameter. The pulley at the top of the shaft is belted to a one-eighth horsepower motor and the water circulated through holes near the top and near the bottom of the cylinder. The cylindrical sides of the boiler and ice container are of gal-

[1] Journal paper no. J 264 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 87.

[2] Gratitude is expressed for the assistance given by Dr. I. E. Melhus in the preparation of this manuscript.

vanized iron lagged with asbestos. The bottom of each container and the top of the boiler are brass plates $\frac{3}{32}$ of an inch in thickness; a hole near the top provides for the insertion of the thermometer.

By means of a cylinder of bakelite co-axially placed, the ice container is divided into two compartments, the outer one, which is about 3 inches wide, serving as a guard ring. Bakelite is used on account of its low thermal conductivity, lessening the possibility of transfer of heat into the cylinder. The cylinder is mounted on a short thin brass ring which is soldered to the bottom of the container. The top of the cylinder is machined out for a short distance down on the inside so as to contain a brass sleeve, which is threaded to mesh with the threads of the edge of the brass disk serving as a lid for the cylinder. The lid is slightly concave on the lower surface and has a hole in its center into which may be fitted a one-holed rubber stopper. Into this stopper is passed a glass tube the upper part of which has two bulbs. Two fine index marks are etched on the tube, one above and the other below the lower bulb. Further details concerning the apparatus may be secured by referring to the original articles.

In series 2 and 3 the oats were not weighed. Whatever oats were needed to fill the space between the two layers were used. In series 1 and 2 the thickness of the layer was 0.94 cm. In part of series 3 the layer was 1.48 cm. in thickness.

The general procedure has been adequately given by the junior writer and need not be repeated here. In order to make the calculations clear, however, it may be well to define some of the terms employed.

In making measurements in c.g.s. units, the specific gravity of ice is taken as 0.9164. For every 0.9164 gm. ice melted in the cylinder there is a volume shrinkage of approximately 0.0836 cc., and the water in the tube would decrease by that amount.

The volume of ice water contained by the lower bulb between the two marks of one of the tubes used was determined and for one of the tubes was found to be 3.87 cc. Therefore $(3.87 \div 0.0836) \times 0.9164$ equals the number of grams of ice melted in the cylinder during the test. With the heat of fusion of ice taken as 79.8 calories per gram, the total quantity of heat, Q, passing into the cylinder during the test was $(3.87 \div 0.0836) \times 0.9164 \times 79.8 = 3385$ calories. This figure is taken as a constant for all tests made with a particular tube. The thermal conductivity, k, of the grain was then readily calculated from the equation

$$k = \frac{Qd}{A\,T\,(t_1 - t_2)}$$

where:

d = thickness of the specimen

A = area under the cylinder

T = time in seconds

t_1 and t_2 = temperatures of the lower and upper surfaces of the specimen

In these studies the temperature of the boiler was maintained at or near 40° C. The moisture content was determined in the usual manner by taking a sample of the oats at the time they were tested. They were subsequently dried in an electric oven held at 100° C. until there was no further loss in weight. In order to bring the oats up to the approximate moisture content desired, a measured amount of water was added to a weighed amount of oats and flasks were shaken at intervals for three days. At the end of this time the moisture was evenly distributed. All determinations were run in duplicate and the average taken.

The oats were of Iowar variety and were grown on the Agronomy Farm the previous year.

A specific example of the calculations used is as follows:

Time—March 4, 1932

200 gm. oats + 20 gm. water Percentage moisture = 16.48

Oats placed in between the boiler and ice compartment 11:00 A.M. Q = quantity of heat passing through; A = area; T = time; d = thickness; k = specific thermal conductivity.

$$Q = \frac{kA\ (\text{time})\ (t_1 - t_2)}{d}$$

$$k = \frac{Qd}{A\ \text{time}\ (t_1 - t_2)}$$

$$Q = \frac{3.87}{0.0836} \times 0.9164 \times 79.8 = 3385 \text{ calories}$$

$$d = 0.94 \text{ cm.}$$

$$A = 122.71 \text{ sq. cm.}$$

$$\text{Time} = 3639 \text{ seconds}$$

Temp. diff. = 40° C.

$$\therefore k = \frac{3385 \times 0.94}{122.71 \times 3639 \times 40} = 0.0001781$$

Volume of tube no. 2 = 3.87 cc.

$$Q = \frac{3.87}{0.0836} \times 0.9164 \times 79.8 = 3385 \text{ calories}$$

I. Time

1:30—Tube 3.87 cc. inserted	40° C.	Temperature of boiler
1:45	40° C.	
2:17—37 (water at top mark of tube)	40° C.	
2:40	40° C.	
3:00	40° C.	
3:18—42 (water column at lower mark of tube)	40° C.	
Time, 1 – 1 – 05 = 3665 seconds	40° C.	

II. Time

3:29—25 (water column at top mark of tube at start)		Temperature of boiler
3:45	40° C.	
4:00	40° C.	
4:15	40° C.	
4:29—38 (water column at lower mark)	40° C.	
Time, 1 – 0 – 13 = 3613 seconds. Average temp.	40° C.	
Average time 3639 seconds		

Experimental data

Three series of experiments are reported in this paper: (1) run January 29 to April 15, 1932; (2) run October 8 to December 16, 1932; and (3) run January 6 to March 10, 1933. The first trial is reported in table I.

TABLE I

THERMAL CONDUCTIVITY OF OATS OF DIFFERENT MOISTURE CONTENTS. SERIES 1, JANUARY 29 TO APRIL 15, 1932

DATE	MOISTURE CONTENT	THERMAL CONDUCTIVITY
	%	
January 29	9.88	0.0001527
February 12	10.51	0.0001650
February 19	11.87	0.0001668
February 26	12.34	0.0001747
March 4	16.48	0.0001830
March 11	25.23	0.0001895
March 18	27.66	0.0001910
March 25	30.32	0.0002014
April 1	34.35	0.0002149
April 8	36.82	0.0002168
April 15	38.38	0.0002220

The oats used in series 1 ranged from 9.88 to 38.38 per cent. moisture content. The thermal conductivity ranged from 0.0001527 to 0.000222. The graph (fig. 1) shows that in general the thermal conductivity increases with the moisture content.

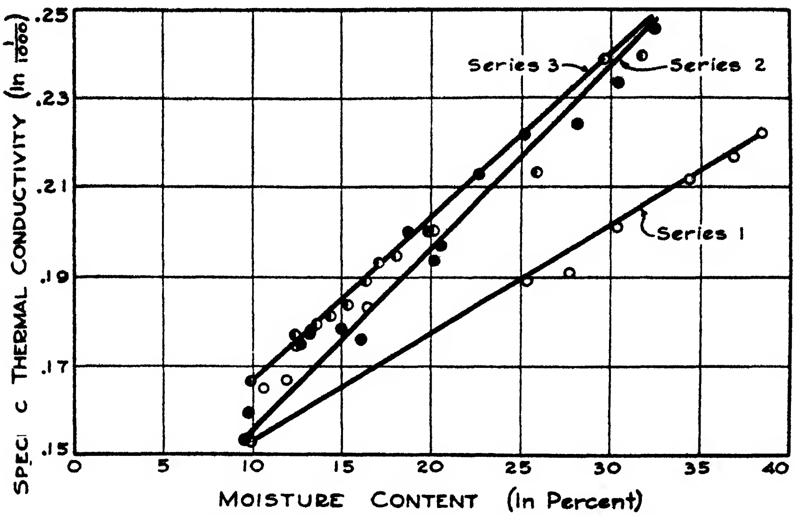

FIG. 1. Increase in specific thermal conductivity of oats with increase in percentage of moisture content.

TABLE II

THERMAL CONDUCTIVITY OF OATS OF DIFFERENT MOISTURE CONTENTS. SERIES 2, OCTOBER 8 TO DECEMBER 16, 1932

DATE	MOISTURE CONTENT	THERMAL CONDUCTIVITY
	%	
October 8	9.54	0.000153
December 2	9.72	0.000159
November 18	12.62	0.000175
November 11	13.183	0.0001771
November 4	14.96	0.000178
October 28	16.09	0.000176
October 21	18.70	0.0002005
November 11	20.14	0.0001938
November 18	20.59	0.0001971
December 2	22.693	0.0002126
December 9	25.107	0.0002228
December 9	28.121	0.0002240
December 16	30.385	0.0002335
December 16	32.49	0.0002455

In table II and figure 1 it is found that in series 2 there is a general increased value in the thermal conductivity when the moisture content is raised. The range in moisture contents is between 9.54 and 32.49 per cent. and the thermal conductivities are found to be between 0.000153 and 0.0002455. The thermal conductivities of the air dried oats are practically

the same in both cases, but the thermal conductivity of the oats with high moisture content is considerably higher than given in table I. In the first series there were 200 gm. of the oats in a layer 0.94 cm. thick; in series 2, the 0.94 cm. layer was filled with oats and leveled off regardless of the exact weight. This apparently produced a more uniformly packed layer. The correlation between moisture content and thermal conductivity became closer.

TABLE III

THERMAL CONDUCTIVITY OF OATS OF DIFFERENT MOISTURE CONTENTS. SERIES 3, JANUARY 6 TO MARCH 10, 1933

DATE	MOISTURE CONTENT	THERMAL CONDUCTIVITY
	%	
February 17	9.877	0.0001671
March 3	12.365	0.0001767
January 6	13.202	0.0001776
February 24	13.50	0.0001701
February 3	14.37	0.0001811
January 20	15.451	0.0001840
January 20	16.324	0.0001893
February 10	17.094	0.0001935
March 10	18.073	0.0001948
February 10	19.873	0.0002000
February 17	20.254	0.0002060
February 24	25.862	0.0002134
March 3	29.640	0.0002388
March 10	31.786	0.0002393

In this series, table III and figure 1, the thermal conductivity of the air dried oats (9.877 per cent.) was 0.0001671, a little higher than in the previous two series. With a moisture content of 31.786 per cent. the thermal conductivity had arisen to 0.0002393. The graph drawn from the lowest moisture content to the highest becomes a straight line.

Discussion

In the data submitted and represented graphically in figure 1, series 2 and 3, the thermal conductivity values increase with the moisture content. In table I, weighed amounts of oats (200 gm.) were used. The tempered grain, that is, that which had had water added, had to be tamped considerably in order to get the weighed amount between the boiler and the ice compartment. This no doubt accounts for the deviation in the moisture contents from 10.51 to 12.34 per cent. In the case of the higher moisture contents the oats did not experience as much increased compression as the oats with lower moisture contents.

In series 2 and 3 the graphs are practically straight, the points are not far removed. In this case the layer of oats was tamped as uniformly as possible but no effort was made to place in this space a known weight of oats.

In table III, series 3, and figure 1, the data bearing dates on and after February 10, 1933, were secured from those where the thickness of the layer of oats used was increased to 1.48 cm. instead of 0.94 cm. as before. Instead of using a tube as large as the one in the other cases the contents were cut to 2.479 cm. It took 350 gm. of air dried oats to fill the space. The thermal conductivity results secured here are in harmony with those secured where the layer of oats was thinner and where the contents of the tube were greater.

It is evident that the moisture increases the thermal conductivity considerably, but even so the rate of heat movement through either dry or damp oats is extremely slow. STILES determined the thermal conductivity of wallboard and found it to be approximately 0.0001150. This value is somewhat lower than that given for dry oats (0.000153). Even with this difference, dry oats makes a rather effective insulating material.

In BAKKE and NOECKER's (1) previous publication on the relation of moisture to respiration and heating, it has been pointed out that "heat pockets" are often formed. These areas are often restricted and well defined and are formed through the rapid oxidation of the grain and associated micro-organisms and the low thermal conductivity of grain even when the moisture content is high. Any method which will dissipate the heat will naturally operate against the low thermal conductivity and produce conditions which will secure a more rapid dissipation of heat. This will make for better storage conditions.

Summary

The thermal conductivity of dry oats has a value of about 0.000153, and increases directly with the moisture content. At a moisture content of 9.88 per cent., the thermal conductivity is 0.000153 and at 38.32 per cent. moisture, 0.000222 in c.g.s. units.

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SULPHUR CONTENT OF PLANTS

John Frazer

Literature review

Although it has been known for more than 70 years that sulphur, together with other elements, is essential to plant nutrition, the question of the sulphur content of plants appears to have received but scant attention. It is significant that numerous investigations are now in progress dealing with the broad question of cell growth, with special reference to sulphur content and to the function of sulphur in such growth. It may well be that more information on the subject of sulphur content and its precise rôle in plant growth will throw light upon certain obscure phases of the development of animal tissue, and eventually become a factor in the solution of the cancer problem.

Before a thorough understanding of the function of sulphur in the growth of plants is possible, more information is essential as to the quantity of this element in plants and what variations in quantity there may be in the course of plant development.

In 1911 Hart and Peterson (7) demonstrated that plant material contained much larger amounts of sulphur than had hitherto been suspected, and that its occurrence in the soil was less than that of phosphorus. Peterson (**12**) showed that sulphur occurs in plant and animal tissues chiefly as protein, volatile compounds (as mustard oils), and as sulphates. He divided the sulphur of plants into four categories, (1) volatile sulphur, (2) sulphates, (3) total soluble sulphur, and (4) insoluble sulphur.

The question of the amount of sulphates in plants has been investigated by Arendt, Ulricht, Schulze, Berthelot and André, Fraps (**6**), and Thompson (**13**), who found wide limits according to the amount in the soil, plant part analyzed, and the stage of growth. Other French investigators (**4**) experimented with sulphur and carbon bisulphide in relation to increased crop production.

Miller (**10**) points out that from 2 to 100 times as much sulphur is needed for the growth of plants as was previously supposed. He divides the sulphur in plants into (1) protein sulphur (cystine), (2) volatile compounds (mustard oil, allyl and vinyl sulphides and mercaptans), and (3) sulphates. Miller states that in some plants 65 per cent. of the sulphur is in the form of sulphates. Plants are incapable of absorbing elemental sulphur. This element is fairly evenly distributed in plants and functions as building material for the formation of protein. Furthermore it increases the root or absorbing system of the plant, the chlorophyll content, and the number of nodules of legumes.

Crocker (5), in discussing the necessity of a sulphur carrier in artificial fertilizers, refers to a general "sulphur hunger" shown by leguminous crops, especially alfalfa, and states that in certain cases the addition of sulphur of any sort increased yields from 500 to 1000 per cent., increases of 50–100 per cent. being common. He adds that the sulphur-treated alfalfa plots of Washington and Oregon show the most striking of the responses to fertilizers. He suggests that the values for the first sulphur analyses of plants were probably low because much of the material was volatilized in the process of ashing.

Crops generally contain about equal amounts of sulphur and phosphorus, but in legumes, cabbage, mustard, potatoes, onions, and chives the sulphur content exceeds that of phosphorus. Sulphur is necessary as a building material. All plant proteins contain sulphur in cystine, and in plant proteins the percentage varies between 0.3 and 7.2 per cent. Apart from proteins, sulphur enters into other constituents of plants as building material, as in mustard oil, mercaptans, allyl and vinyl sulphides of the onion and garlic. Sulphur is also important in the formative effects in plants, and has been observed to produce a great increase in size and extent of the root system of alfalfa, as well as a marked nodule development in this plant (Ritz) and also in red clover (Miller). In agreement, Crocker and Miller (10), state that sulphur assists chlorophyll development, causes increased root or absorptive systems in legumes, increased nitrogen fixing apparatus and carbohydrate manufacturing apparatus. Crocker suggests that it may be of great significance in supplying building materials for sulphur-rich animal tissues, *e.g.*, hoofs, hair, and nails. Animal proteins, he states, are generally richer in sulphur than are plant proteins. He points out further that sulphur fertilizer ($CaSO_4$) greatly increases yields of cotton which, just before blooming time, show a content of sulphur three times as much as of phosphorus. Increases in sugar cane crops have been produced by Calvino in Cuba, using $CaSO_4$ as fertilizer.

Marsh (9) states that the sulphur content of apple trees is of the same order as that of phosphorus. The sulphur curve is correlated directly with variations in pH. Sulphur in tomato tissue was 2½–4 times the amount of phosphorus, and the nitrogen content doubled that of sulphur. The older the tissue the higher was the sulphur content. In cases where sulphur is high, phosphorus is low and the amounts seemed to run inversely to each other, just as in the case of apple tree tissues. It has been suggested that sulphur may liberate phosphorus from the older tissues for translocation to the younger growing tissue. Sulphur seems to parallel pH. Rosa found the pH in the tomato to have the following values:

in plant tips	5.7
in fruiting region	4.8

As the same correlations were found in the apple tree tissues, there must exist some fundamental relationship between sulphur, pH, and phosphorus. Fertilizer treatments caused much variation in the amounts of phosphorus and sulphur present. The addition of acid phosphate markedly increased the sulphur and phosphorus content.

In his paper on the sulphur requirement of the red clover plant, TOTTINGHAM (14) states that HART and TOTTINGHAM reported increased production of dry matter by plants that had received $CaSO_4$ and Na_2SO_4 in soil culture. Red clover gave greater response to $CaSO_4$ than to Na_2SO_4. RITZ found the development of red clover bacteria to be stimulated by the sulphur from $CaSO_4$. Magnesium sulphate was found to be inferior to sodium sulphate or calcium sulphate for red clover in water or sand culture. $CaSO_4$ proved to be especially efficient. It was found that a deficiency of sulphur restricted growth by limiting the synthesis of protein.

TABLE I

SULPHUR CONTENT OF LEAVES

(PERCENTAGE OF DRY WEIGHT OF PLANT MATERIAL)

PLANT	SULPHUR CONTENT				
	MINIMUM	MAXIMUM	AVERAGE	NO. OF SPECIMENS	BOTANIC GARDEN, U. OF P.
	%	%	%		%
Picea	0.030	0.135	0.083	32	
Pinus	0.000	0.365	0.105	47	0.440
Tsuga	0.050	0.260	0.138	10	0.450
Abies	0.025	0.260	0.117	20	0.245
Taxus	0.015	0.125	0.073	15	0.350
Chamaecyparis	0.075	0.180	0.120	17	0.350
Thuja	0.030	0.110	0.051	14	.....
Juniperus	0.065	0.115	0.083	17	...
Cryptomeria	0.105	0.105	0.105	3	0.267
Cephalotaxus	0.120	0.160	0.140	2	0.430
Cupressus	0.185	0.185	0.185	11	......
Thujopsis	0.060	0.075	0.065	3	......
Sciadopitys	0.335	0.410	0.370	3	..
Rhododendron	0.100	0.275	0.161	4	0.180
Buxus	0.090	0.365	0.191	30	0.390
Mahonia	0.105	0.105	0.105	1	0.315
Hedera	0.080	0.140	0.120	4	0.490
Kalmia	0.008	0.075	0.042	2	....
Bambusa	.	...	0.295	1	0.353
Aucuba	0.475	0.475	0.475		0.475
Magnolia	0.030	0.130	0.080	2	....

It is interesting and significant to note that workers in this field are in agreement as to the influence of sulphur upon growing plants with respect to the synthesis of protein. MILLER, CROCKER, and TOTTINGHAM are apparently in entire accord on this point.

Much has been written on the question of injury to vegetation by sulphur compounds (**11**), especially by SO_2. It has been found (**16**) that very minute quantities of SO_2 acting for long periods of time are unable to disturb the normal growth of plants. Injury from smelter smoke, which has been the subject of extensive litigation, is greater immediately after irrigating the soil or after rain. The effects of such injury are more noticeable in the case of crops whose leaves are near the ground than when the leaves grow higher, and, as might be expected, young plants and their young tender parts are more seriously affected than older and hardier plants or parts.

BRIZI (**3**) found that injury to plants takes place from the exterior and not through the roots as a medium. If this be a correct assumption, the absorption of sulphur compounds from the atmosphere at once assumes added significance and importance.

The investigation here reported concerning the sulphur content of plants included particularly a study of the degree to which plants are capable of absorbing sulphur from prevailing atmospheric gases. It will be observed,

TABLE II

SEASONAL VARIATION IN SULPHUR CONTENT (PERCENTAGE OF DRY WEIGHT OF PLANT MATERIAL)

PLANT	MAY 30, 1932 (OUTDOOR ARTS CO.)	NOVEMBER TO JANUARY, 1933 (MORRIS ARBORETUM)	
	%	%	
Pinus mugo	0.050	0.050	November 28, 1932
Pinus austriaca	0.115	0.045	
Pinus flexilis	0.115	0.070	
Pinus densiflora	0.095	0.075	
Chamaecyparis obtusa	0.075	0.135	January 30, 1933
Chamaecyparis crippsii	0.100	0.105	
Chamaecyparis filifera	0.110	0.080	
Chamaecyparis squarrosa	0.145	0.150	
Chamaecyparis pisifera	0.090	0.160	
Chamaecyparis plumosa	0.165	0.100	
Taxus baccata	0.090	0.060	
Taxus cuspidata	0.085	0.020	
Tsuga canadensis	0.170	0.195	
Thuja occidentalis	0.040	0.010–0.050	
Juniperus chinensis	0.090	0.100	
Juniperus argentea	...	...	
Juniperus pfitzeriana	0.080	0.070	
Average	0.101	0.095–0.098	

TABLE III
SULPHUR CONTENT OF LEAVES
(PERCENTAGE OF DRY WEIGHT OF PLANT MATERIAL)

Plant		Chestnut Hill, Pa.
		%
Deciduous trees	*Hydrangea*	0.570
	Lilac	0.100
	Apple	0.050
	Privet	0.330
	Maple	0.225
Herbaceous herbs	Tobacco leaves	0.360
	Violet sp.	0.185
	Cauliflower stem	0.495
	Jonquil leaves	0.170
	Convallaria leaves	0.135

in the tables which are presented here, that there is considerable difference, sometimes marked, in the sulphur content of plants of the same kind when taken (a) from a locality in which the atmosphere is free from sulphurous gases; and (b) from a locality where these gases are prevalent in varying concentrations, as for example, the central section and suburbs of a large manufacturing city where smoke and gases pollute the air.

Investigation

Comparisons of analogous species from different localities were made. The analyses reveal but slight variations in sulphur content, whether the specimens were taken during the dormant season or during the period of active growth.

PROCEDURE.—Each sample was first freed from dust and any mechanically adhering matter. It was then carefully dried in a Freas electric oven at a temperature not exceeding 108° C. The leaves or needles were then separated from the woody part of the branch and bottled in glass-stoppered bottles to prevent the absorption of moisture or the effects of laboratory fumes.

For each analysis approximately 2 gm. of the material were used, the weight being taken on a delicate balance. The sample was placed in a porcelain evaporating dish, covered with distilled water, and 10 cc. of pure nitric acid (sp. gr. 1.42) added. The evaporating dish was then placed on a water bath for 2.5–3 hours until evaporated to complete dryness. To the residue were added about 40–50 cc. of boiling water and 2–3 drops of concentrated hydrochloric acid in order to dissolve any soluble matter. This was then filtered into a beaker through no. 42 Whatman's filter paper and the precipitate washed thoroughly with boiling water.

The filtrate was then brought to boiling and a slight excess of barium chloride solution added while stirring. The precipitate and solution were covered and allowed to stand for at least 12 hours, usually overnight, to permit the precipitate of barium sulphate to settle completely.

The solution was again filtered and the precipitate carefully washed with boiling water, dried, and ignited in a weighed Sillimanite porcelain crucible which was then placed in a desiccator for several hours before weighing. From the weight of the barium sulphate the weight of sulphur was calculated, and from the latter the percentage in the original sample was determined.

Conclusion

The results indicate that certain species of coniferous plants and others obtained from localities where there is distinct atmospheric contamination from factories, chimneys, etc. contain several times as much sulphur as corresponding species from a region where the influence of such atmospheric pollution is nil, or so slight as to be negligible.

To the Director of the Botanic Garden and of the Morris Arboretum of the University of Pennsylvania, from which sources numerous specimens were supplied for this investigation, the writer expresses his grateful appreciation.

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BIOCHEMICAL PHASES OF OLEORESIN PRODUCTION[1]

ELOISE GERRY AND J. A. HALL

(WITH FOUR FIGURES)

The key position of naval stores production in the profitable utilization of the more than 50,000,000 acres of longleaf and slash pine lands of the southeastern United States puts a premium upon exact and comprehensive knowledge of the biochemical facts which underlie successful oleoresin production. Oleoresin, or gum, the source of naval stores, exudes from cuts on living pines, from their earliest seedling stage. A cut into a young sapling, for example, illustrates the abundance of the exudation. The exudate is not the sap of the tree in the ordinary sense of the term, however, nor does it come from the ordinary sap-conducting tissues as does the watery solution of the exuding maple sap.

The exudation of oleoresin comes from areas of parenchyma cells in the outer sapwood next to the bark (fig. 1). These parenchyma cells contain active functioning protoplasm for a longer period than the associated fibrous cells which conduct the watery sap. The parenchyma cells also manufacture, transform, translocate, and store many different compounds, including sugars, tannins, and starch. Resin passages, the so-called resin ducts, are spaces which arise in the midst of clusters of parenchyma cells by a separation or splitting apart of the cells (schizogenous not lysigenous origin). It is from these that the oleoresin collected in turpentining wells out in drops (fig. 2, *A*). The chipping practiced by the turpentine operator is a freshening of the wound or cut made in the sapwood, which causes a continued and even augmented exudation of oleoresin.

Resin passages are an outstanding characteristic of all pine wood, but they reach an optimum development in slash (*Pinus caribaea*) and longleaf (*P. palustris*) pines. They extend both vertically and horizontally in the tree. The two systems frequently connect with each other and with the rays (also parenchymatous in nature) at different levels, thus forming an extensively anastomosing system which is also connected with the parenchyma tissues of the phloem or inner bark through which compounds elaborated in the leaves are translocated (fig. 1).

Resin passages are more numerous than is often realized. Recent microscopical investigations at the Forest Products Laboratory have shown (7)

[1] Contribution from the Forest Products Laboratory, maintained at Madison, Wisconsin, in cooperation with the University of Wisconsin.

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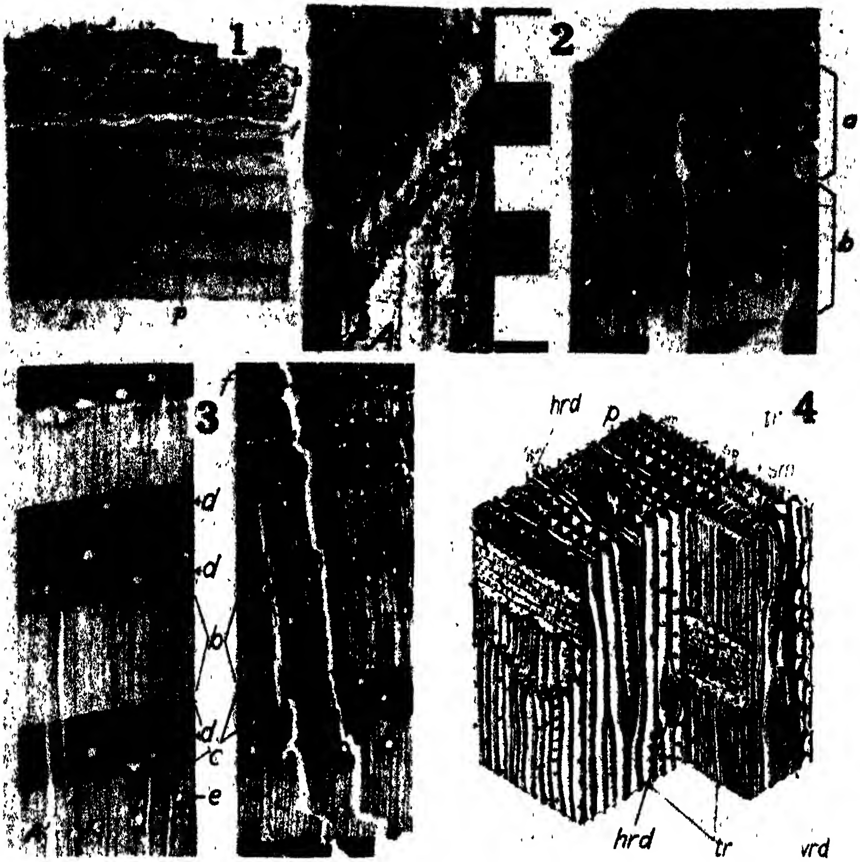

FIGS. 1–4. Fig. 1: Microscopical view of cross section of pine wood showing vertical resin passages (*p*) at center of groups of parenchyma cells; rays (*r*), including a wide ray with a horizontal resin passage connecting a vertical resin passage with the inner bark or phloem (*b*) rich in stored material; and the wood tracheids or fibers (*f*) which function in water conduction. Fig. 2: *A*, heavy exudation next bark obtained from the increased number of wound-induced resin passages; *B*, wood above the face or wound on a turpentined pine; *b*, normal wood formed before turpentining; *a*, two years of growth during turpentining showing great increase in number of resin passages. Fig. 3: *A*, cross section of normal southern pine wood (slash pine); *a*, portion of one annual growth ring; *b*, springwood; *d*, summerwood; *e*, resin passages; *B*, wood formed by same tree over face during turpentining, containing greatly increased number of resin passages; *a*, portion of one annual growth ring. Fig. 4: Diagrammatic drawing of pine wood structure showing horizontal resin passages (*hrd*) and vertical resin passages (*vrd*) and the connections frequently found between them. Also springwood fibers (*sp*), summerwood fibers (*sm*), and rays (*wr*, *fwr*).

that in slash pine on the average more than 450 horizontal resin passages were present per square inch of tangential surface (fig. 4). The number present in various specimens ranged from about 250 to 800 per square inch. The exudation from these horizontal resin passages is more directly utilized in the French method of turpentining than in the American, because a relatively greater area of tangential surface is regularly freshened owing to the shape of the French wound or face.

The vertical resin passages, which are much larger than the horizontal passages, number about 200 per square inch of cross section in normal wood. This number ranges from about 70 to 400 per square inch. In wood formed above the face during turpentining the number of vertical resin passages may be increased in varying amounts up to more than tenfold the normal number (fig. 3). The induced increase of resiniferous tissues by wounding is of considerable importance since a large proportion of the yield obtained after the first year of turpentining comes from it (fig. 2, *A*). The effect of the wound may extend vertically for 10 to 20 feet above the face and is clearly registered in the structure of the wood formed above the face. The wound response is limited to a few inches circumferentially, however, although characteristic traumatic structures are apparent in the rolls of healing tissue formed at the sides of the face. They often offer a rich source of oleoresin when trees are back-cupped during successive workings.

Investigations other than chemical have dealt to date with anatomical and physiological studies of the normal character of resiniferous tissue in virgin and second-growth trees, and with the responses induced by different types of wounding. The work now in progress is a joint attack, using in addition cytological, microchemical, and biochemical techniques to determine the character and sequence of changes in the compounds which underlie oleoresin formation.

Oleoresins are biological materials, products of the living protoplasm, and therefore referable to the original products of photosynthesis for their ultimate chemical derivation. It must be said that here, however, as with most other complex substances of vegetable origin, not one step is yet known with certainty in the complex processes by which the pine tree elaborates oleoresin. But if empirical experimentation in forest management and turpentining is to be firmly grounded, all possible information must be obtained concerning the biochemistry of oleoresin formation.

Although technologically pine oleoresin has usually been considered as merely a solution of resin acids in a mixture of terpenes, such a concept does not suffice in an investigation of this kind. As a product of vital processes, oleoresin might be expected to contain small quantities of materials not yet completely elaborated, the careful examination of which may throw considerable light on the processes immediately preceding the excretion of the oleo-

resinous mixture. A part of this investigation therefore involves the thorough examination of substances in the oleoresin other than terpenes and resin acids, particularly such materials as are soluble in water.

The place of excretion of the completed oleoresin is known, as has been indicated (fig. 2), although the mechanism of the process is unknown. Similarly the processes by which assumed complex oleoresin precursors are translocated are unknown, as well as the seat of formation within the tree of these assumed complexes. A second phase of this investigation, therefore, involves the chemical examination of the tissues of the pine which are apparently concerned in oleoresin formation. A definite search is being made for substances which may be defined as oleoresin in process of formation.

Such a concept is by no means original, although comparatively little evidence has hitherto been brought forward in support of it. KÖHLER (**8**) found certain resinous secretions in *Picea excelsa,* which were subject to rapid and spontaneous change upon exposure to the air. He postulated an aldehydic or camphoraceous precursor, $C_{10}H_{16}O$, which was secreted as such, and changed to the mixture of resin acids and terpenes outside of the living cells.

DUPONT (**4**) developed an ingenious formulation, *viz.,*

$$3 \quad C_{10}H_{16}O = C_{10}H_{16} + C_{20}H_{30}O_2 + H_2O.$$

The elaboration of this equation explains very satisfactorily the proportions of resin acids and terpenes found in the oleoresins from *Pinus maritima* and *P. halepensis,* in which the ratio of rosin to turpentine approximates closely the 69 : 31 ratio required by DUPONT's equation. However, numerous pines do not approximate this composition in their oleoresins; slash and longleaf oleoresins run more nearly a ratio of 80 : 20 (**1**). It is also true that there is appreciable variation in turpentine content between individual trees. Further, it must be considered that any such mechanism must also explain the fact of the existence of n-heptane as the volatile ingredient of oleoresins from *P. jeffreyi* and *P. sabiniana,* as well as the large proportions of sesquiterpene in the oleoresin of *P. longifolia.* In short, although the assumption of some common precursor of volatile and non-volatile components of oleoresin has a logical basis, it does not seem that so simple a balance as indicated by DUPONT can explain the facts of oleoresin composition throughout the genus *Pinus.*

DEVAUX and BARGUES (**3**) showed that the oleoresinous materials were not entirely localized in the resin passages of the wood and in the cells bordering them. The living parenchyma cells of the wood and phloem are connected with the resin passages through the rays (fig. 2) which allow translocation of the materials which are concerned in oleoresin production. Noteworthy is the statement of DEVAUX and BARGUES that the secretion is acidic

only in the canals and generally neutral in the living cells. The change is said to occur when the resin reaches the canal, although it may occur prematurely.

Glichitch and Naves (5) call attention to the difference in sesquiterpene content of ylang-ylang oils obtained by steam distillation and petroleum ether extraction. The very low content of sesquiterpenes in the latter leads them to suggest that the sesquiterpenes may arise during the distillation process from substances insoluble in petroleum ether and non-volatile with steam.

There is further the evidence of Bridel (2) concerning the existence of glucosides of geraniol, which gives a clue to the possible nature of the mechanism by which substances insoluble in cell sap are held in solution by means of glucosidal combinations.

A recent theoretical study (6) showed the probable derivation of the terpenes from underlying hydroxylated bodies. A similar type of generalization doubtless holds for the resin acids although sufficient examples of known structure are not yet available to warrant their classification. Such assumptions seem more warranted than the well known isoprene polymerization in that nothing has been observed indicating the existence of isoprene in nature, nor has it been formed *in vitro* by means analogous to those of biological reactions.

From these considerations, the search for oleoresin precursors both in the oleoresin and in the tissues of the tree has been primarily for glucosidal materials, water-soluble and resolvable into oleoresinous constituents and sugars. The difficulties of this work are apparent, since it involves the isolation of very small quantities of delicate compounds from very large quantities of starting material by means sufficiently gentle to avoid decomposition. While no single phase of the problem may be regarded as completed, considerable progress has been made which is briefly reported here.

The oleoresin itself contains small quantities of highly complex glucosidal material which is soluble in water and can be obtained by treatment of the oleoresin with water. The original clear, light, aqueous extract soon deposits a black, amorphous precipitate which is now insoluble in water but soluble in alcohol. This substance is easily hydrolyzed, yielding a large proportion of acidic bodies of a resinous nature. While these substances cannot yet be identified, since they have been available to the extent of only a few grams, their color reactions are those of the acids of rosin, and it is extremely probable that they belong to this class of substances.

The solution from which this dark precipitate has been obtained still contains a considerable quantity of material in solution. By extraction with ether, a substance is obtained which is instantly hydrolyzed by very dilute acid. It yields a resin acid, a volatile oil, and a water-soluble body. The

last may be broken up further into a sugar and a resin acid. The sugar is remarkable in that it reduces Fehling's solution in the cold, and reacts with phenylhydrazine very quickly in the cold, yet it is not an uronic acid. It is highly reactive and tends to polymerize to unworkable complexes on very slight provocation.

Complete interpretation of these results is still far distant, and must await considerably more material and effort. However, they suggest very definitely the presence in the oleoresinous exudate of glucosidal complexes of resin acids, volatile oil, and reactive sugars, very easily broken up and very sensitive to slight changes in environment.

Investigation of slash pine tissues has so far been confined to the phloem, since, as was pointed out, this seems to constitute a rich reserve tissue (fig. 2), physiologically closely related to that immediately concerned with production of the oleoresin in the resin passages of the wood or xylem. While a rather complete phytochemical survey of this material has been made, it will suffice in this connection to point out only the occurrence in the phloem of glucosidal complexes of exactly similar nature to those obtained from the oleoresin itself; that is, a glucoside soluble in both water and ether, yielding to very mild acidic hydrolysis in the cold, resin acid, volatile oil, and the same resin acid-sugar complex as obtained from the oleoresin.

It is regretted that further data on the constitution of these substances cannot be given at this time, but their extremely complex nature makes necessary the preparation of further quantities of material for more complete investigation. It is believed, however, that the attack thus far has been justified and has yielded considerable chemical substantiation of the anatomical evidence of functional connection between resiniferous tissues of both phloem and xylem, and thrown considerable light on the processes preceding the excretion of oleoresin at the wounded surface.

At present the picture presented is of a large complex of resin acids and terpenes combined with sugars, the whole being thus soluble in the elaborated sap of the parenchyma cells and translocated as such to the neighborhood of the area of excretion, where it is resolved by a mechanism still obscure.

In conclusion, it may be pointed out that the demonstrated presence of these highly reactive, water-soluble bodies in the oleoresin contributes in no small degree to the variations in color of manufactured rosins, and is thus of considerable technological importance.

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SOME EFFECTS OF THE PHOTOPERIOD ON DEVELOPMENT OF *IMPATIENS BALSAMEA*[1]

J. PERRY AUSTIN

(WITH TWO FIGURES)

Introduction

In 1920 GARNER and ALLARD (**1**) discussed the influence of length of the daily light period on flowering. A later paper by the same investigators (**2**) recorded further observations of its effect on flowering and other phases of plant development.

GARNER and ALLARD (**1**) proposed two terms, *photoperiodism,* "to designate the response of an organism to the relative length of day and night," and *photoperiod,* "to designate the favorable length of day [for flowering] for each organism." *Photoperiodism* appears frequently in the literature on the subject. Shortly after the term *photoperiod* was proposed, however, it was observed that the length of the daily light period also influences phases of plant development other than reproduction. The term thus became unserviceable and apparently has never been used since. To make it useful and to give it a logical relation to the term *photoperiodism,* it is therefore suggested that *photoperiod* be used to designate the daily period of illumination under the influence of which an organism is developing. It is so used in this paper.

The experimental work here reported was carried on from the middle of March to the first of August, 1932. Plants of *Impatiens balsamea,* variety White Perfection, were grown in pots in the greenhouse and were subjected to photoperiods of 8, 16, and 20 hours, and normal. The normal photoperiod (sunrise to sunset) during the experiment ranged from 12 hours at the beginning to approximately 15 hours at the end. The plants were all kept in one section of the greenhouse during the day to secure as nearly uniform conditions of light intensity and temperature as possible. Eight hours after sunrise one lot of plants was removed to a ventilated darkroom until after dark when they were returned to their original places. Late in the afternoon two lots were removed to other sections of the greenhouse where they received electric illumination from sunset until 8 hours and 4 hours before sunrise, giving 16- and 20-hour photoperiods respectively. The intensity of the artificial light was approximately 125 foot-candles at the apices of the plants, as measured by the MacBeth illuminometer. The plants receiving artificial light were returned to their original places early in the morning. During the cooler weather at the beginning of the experiment the tempera-

[1] Paper from the Department of Botany, University of Michigan, no. 467.

ture was kept between 18° and 23° C., day and night; during the late spring and summer, however, the temperature often rose to 30°–35° C. about noon. The temperatures of the darkroom and auxiliary sections were approximately the same as in the main compartment.

Investigation

In general, the plants subjected to the 8-hour photoperiod appeared much less vigorous vegetatively than did those under the longer photoperiods. The flowers and seed pods were about half the size of the others. The seeds produced by the former were of normal size, but there were fewer seeds per pod than under the longer photoperiods.

Effect of length of photoperiod on leaf area

At intervals during the experiment, certain leaves were removed from groups of ten plants each from those growing under the various photoperiods. Blue-prints were made of the leaves and their areas were measured with a planimeter. The average areas are tabulated in table I. The "first leaf" is the lower of the two plumule leaves.

TABLE I

Area in square cm. of certain leaves from plants of *Impatiens balsamea* grown under various photoperiods

Age of plants	Length of photoperiod			
	8 hours	Normal	16 hours	20 hours
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*
	Cotyledons			
14 days	2.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	3.2 ± 0.1
27 days	1.8 ± 0.1*	2.8 ± 0.3*	3.1 ± 0.3	3.8 ± 0.1
	Plumule leaves			
14 days	1.4 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	3.3 ± 0.1
27 days	3.0 ± 0.1	6.9 ± 0.2	7.6 ± 0.3	7.7 ± 0.3
	First leaf			
41 days	3.4 ± 0.2	6.3 ± 0.4	6.4 ± 0.3	6.1 ± 0.2
55 days	3.6 ± 0.1	6.2 ± 0.4*	6.4 ± 0.4	5.7 ± 0.2*
	Fifth leaf			
41 days	5.2 ± 0.4	10.2 ± 0.4	11.2 ± 0.5	8.3 ± 0.6
55 days	4.9 ± 0.3*	9.8 ± 0.3*	12.5 ± 0.6	9.8 ± 0.4
	Tenth leaf			
55 days	....	8.1 ± 0.5	9.7 ± 0.7	9.8 ± 0.7

* The areas given are averages of leaves from ten plants of a particular age. The areas given for the leaves from plants of different ages are based on averages of different plants; hence the apparent decrease in area in the starred instances.

From the data it is clear that the differences between the areas of leaves developed under the three longer photoperiods are not sufficiently great to be significant. On the other hand, in every instance the leaves developed under the 8-hour photoperiod are markedly smaller than the others.

Effect of length of photoperiod on budding and flowering

The plants were examined daily, and the dates when the flower buds first became visible and when the first flower opened on each plant were recorded. The data on age at budding are tabulated in table II. The columns headed "frequency" indicate the number of plants on which flower buds appeared at the age in question.

TABLE II

Age at which flower buds appeared on plants of *Impatiens balsamea* grown under various photoperiods. Columns headed "frequency" indicate number of plants on which buds appeared at the age in question

Length of photoperiod							
8 hours		Normal		16 hours		20 hours	
Age	Frequency	Age	Frequency	Age	Frequency	Age	Frequency
19	1	20	3	56	2	55	1
20	9	21	4	57	5	56	3
21	15	22	24	58	6	57	1
22	38	23	33	59	18	58	11
23	12	24	12	60	3	59	9
24	1	25	4	63	1	60	3
25	1	26	1	65	3	61	1
27	1			66	1	66	1
				68	1	68	2
				70	1	70	2
				72	2	76	2
				76	2	78	3
				78	5	87	2
						90	1
Average age at budding						91	1
21.8 ± 0.1		22.8 ± 0.1		62.7 ± 0.7		64.5 ± 0.95	

The average ages at budding under the 8-hour and under the normal photoperiods do not differ significantly. The same is true of the average ages at budding under the 16- and 20-hour photoperiods, budding occurring considerably later than under the two shorter photoperiods.

Table III contains the data on age at flowering. The average ages at flowering under the various photoperiods bear the same relation to one another as do the ages at budding. Furthermore, a comparison between the average ages at budding and at flowering (table IV) shows that the number

TABLE III

AGE AT WHICH FIRST FLOWER OPENED ON PLANTS OF *IMPATIENS BALSAMEA* GROWN UNDER VARIOUS PHOTOPERIODS

LENGTH OF PHOTOPERIOD							
8 HOURS		NORMAL		16 HOURS		20 HOURS	
AGE	FREQUENCY	AGE	FREQUENCY	AGE	FREQUENCY	AGE	FREQUENCY
43	3	43	1	62	1	77	1
44	1	44	1	67	1	84	4
45	4	46	4	75	1	86	2
46	5	47	7	85	3	87	11
47	9	48	7	87	14	90	2
48	3	49	15	89	2	91	4
49	8	50	10	91	4	92	1
50	6	51	6	95	1	93	1
51	4	52	2	97	1	99	2
52	1	53	3	101	1	100	1
53	4	54	1	103	1	106	1
54	5	57	2	106	1	108	1
55	1	59	1	109	1	115	1
56	2	61	1	110	1	125	1
58	1			111	1	132	1
				112	1	136	1
				115	1		
				130	1		
Average age at flowering							
49.2 ± 0.3		49.6 ± 0.3		92.0 ± 1.4		92.8 ± 1.6	

of days from budding to flowering is practically the same under all four photoperiods. This indicates that in *Impatiens balsamea* the effect of the length of the photoperiod with respect to the reproductive phase of development is on the initiation of the flower buds, the development of the flower from the bud being unaffected.

TABLE IV

RELATION BETWEEN AVERAGE AGE AT FLOWERING AND AT BUDDING

	LENGTH OF PHOTOPERIOD			
	8 HOURS	NORMAL	16 HOURS	20 HOURS
Age at budding (in days)	21.8 ± 0.1	22.8 ± 0.1	62.7 ± 0.7	64.5 ± 0.95
Age at flowering (in days)	49.2 ± 0.3	49.6 ± 0.3	92.0 ± 1.4	92.8 ± 1.6
Days from budding to flowering	27.4 ± 0.3	26.8 ± 0.3	29.3 ± 1.6	28.3 ± 1.9

The facts that the average age at budding under the 8-hour photoperiod is the same as under the normal, and that the average age at budding under the 16-hour photoperiod is the same as under the 20-hour, suggest that somewhere between 12 and 16 hours there must be a critical length of photoperiod.

Effect of length of photoperiod on development of main axis

The height of the main axis was measured at approximately weekly intervals. The average heights at definite ages are tabulated in table V together with the number of plants upon which each average is based. The data are presented graphically in figure 1. The average ages at budding and at

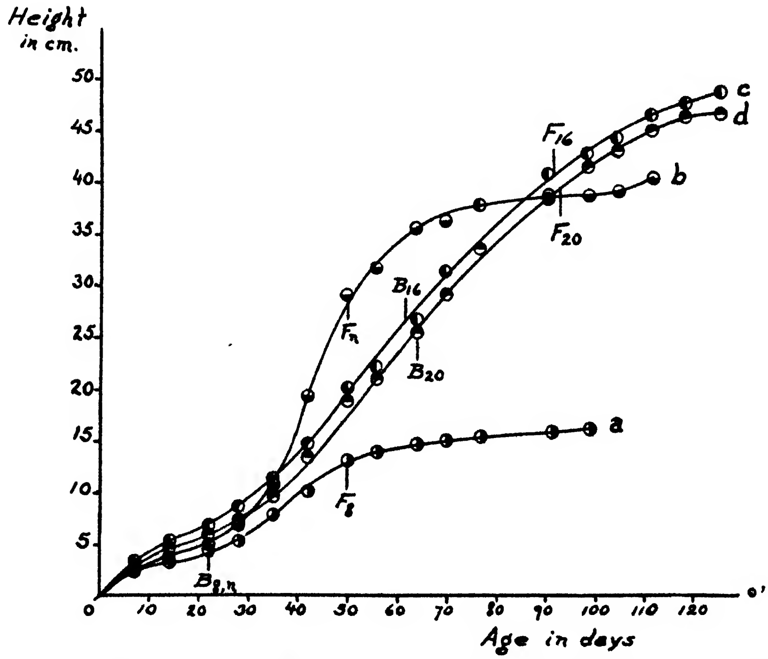

FIG. 1. Relation between length of photoperiod and development of main axis in terms of average height at weekly intervals. Curves *a*, *b*, *c*, and *d* represent average heights of plants grown under 8-hour, normal, 16-hour, and 20-hour photoperiods respectively. $B_{8,\,n,\,16,\,20}$ indicate time of budding (average) under respective photoperiods. $F_{8,\,n,\,16,\,20}$ indicate time of flowering (average) under the corresponding photoperiods.

flowering are also indicated. The height attained is greater under successively longer photoperiods up to 16 hours; a 20-hour photoperiod resulted in slightly but consistently shorter plants than those under the 16-hour photoperiod, which must therefore be approximately the optimum for development of the main axis.

A consideration of the average height attained at various ages relative to the height at budding (fig. 2) emphasizes the fact that at the time the

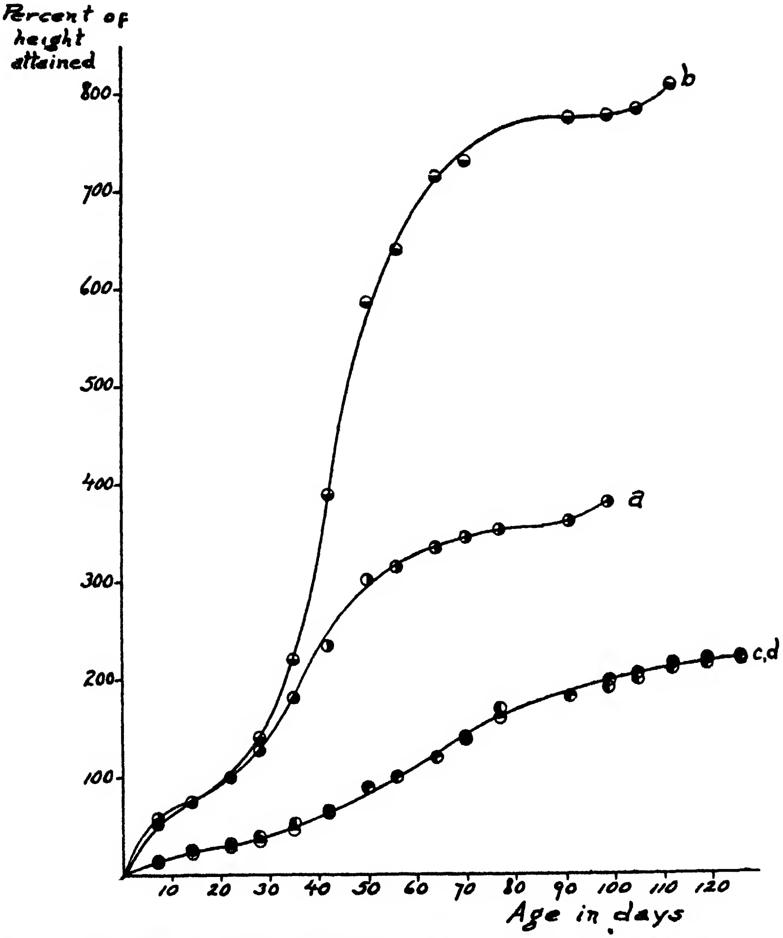

FIG. 2. Percentage of height attained at weekly intervals on basis of average height at budding (average) = 100 per cent. Curves *a*, *b*, *c*, and *d* represent percentage height at budding attained by plants grown under 8-hour, normal, 16-hour, and 20-hour photoperiods respectively.

flower buds appeared the plants grown under the 8-hour photoperiod had attained approximately one-fourth of their total height; those under the normal photoperiod, one-eighth; and those under the 16-hour and 20-hour photoperiods, one-half of their total height. This seems to indicate a considerable degree of independence between the effect of the length of the photoperiod on stem development and its effect on budding.

Discussion

With respect to the effect of the length of the photoperiod on flowering, the preceding data indicate that *Impatiens balsamea* is intermediate between

TABLE V

AVERAGE HEIGHT, AT WEEKLY INTERVALS, OF PLANTS OF *IMPATIENS BALSAMEA* GROWN UNDER VARIOUS PHOTOPERIODS

AGE	LENGTH OF PHOTOPERIOD							
	8 HOURS		NORMAL		16 HOURS		20 HOURS	
	NO. OF PLANTS	AVERAGE HEIGHT	NO. OF PLANTS	AVERAGE HEIGHT	NO. OF PLANTS	AVERAGE HEIGHT	NO. OF PLANTS	AVERAGE HEIGHT
days		*cm.*		*cm.*		*cm.*		*cm.*
7	92	2.6	94	2.6	93	3.3	89	2.9
14	90	3.3	91	3.8	92	5.5	87	4.7
22	76	4.4	79	5.0	80	7.0	74	6.1
28	68	5.6	71	7.1	72	8.9	65	7.4
35	67	8.0	71	11.0	72	11.4	65	9.7
42	57	10.3	61	19.5	62	14.8	55	13.5
50	55	13.3	59	29.4	60	20.2	53	19.0
56	47	13.9	51	32.1	52	22.3	45	21.1
64	41	14.8	46	35.8	47	26.8	40	25.6
70	46	15.2	51	36.6	50	31.4	45	29.2
77	25	15.5			29	38.1	28	33.8
91	36	15.9	40	38.8	40	40.9	40	38.4
99	26	16.3	29	38.9	30	42.9	30	41.7
105			28	39.2	30	44.4	30	43.2
112			25	40.5	29	46.7	30	45.1
119					17	47.8	15	46.4
126					13	48.8	15	46.8

the long-day and short-day types of plants which flower only under relatively long and short photoperiods respectively.

Although the data suggest a number of lines of speculation, it seems best to defer extensive interpretations until similar data from other plants are available.

Summary

Plants of *Impatiens balsamea* were grown under 8-hour, normal, 16-hour, and 20-hour photoperiods and data obtained on leaf area, age at budding and flowering, and development of the main axis. The data bring out the following facts in regard to the response of this species to the length of the photoperiod:

1. The leaf area is approximately the same under the three longer photoperiods but is much less under the shortest one.
2. The effect of the length of the photoperiod on the time of flowering is primarily through its effect on the initiation of flower buds.
3. There must be a critical length of photoperiod, between 12 and 16 hours, with respect to the age at which flower buds are initiated.

4. The optimum length of photoperiod for stem development is apparently about 16 hours.

5. The effect of the length of the photoperiod on budding seems to be independent of the effect on stem development.

This work was done under the direction of Dr. F. G. Gustafson.

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FACTORS RENDERING THE PLASMOLYTIC METHOD INAPPLICABLE IN THE ESTIMATION OF OSMOTIC VALUES OF PLANT CELLS[1]

ELIZABETH C. M. ERNEST

(WITH TWO FIGURES)

In 1884 DE VRIES (**10**) introduced a new method, that of determining the osmotic value of plant cells by immersion in solutions of known concentrations of cane sugar or potassium nitrate. He determined the concentration of each solute which would bring about *plasmolysis* or the withdrawal of the protoplasm from the walls of the cells. He reasoned that all solutions inducing the same minimum degree of visible plasmolysis of cells must be of equal strength. Various aspects of the phenomenon of plasmolysis have been elaborately studied since then by DE VRIES (**11**), FITTING (**4**), HANNIG (**5**), ILJIN (**8**), HÖFLER (**6, 7**), URSPRUNG and BLUM (**9**), BECK (**1, 2**), WEBER (**12, 13**), and many others. These workers have interpreted the results obtained by this method as furnishing adequate data for the estimation of the osmotic value of the cell sap.

Objection may be raised to this interpretation on two grounds. In the first place, the material used by DE VRIES, and by most of his followers, cannot be considered to have been in normal, uninjured condition. Thin sections were prepared for microscopic examination by sectioning, thus subjecting the cells to the deleterious osmotic and other actions of sap exuded from adjacent ruptured cells. That this influence may be important has been pointed out elsewhere (**3**). Moreover, the phenomenon commonly designated as plasmolysis, although usually regarded as purely osmotic in nature, may be induced by several other causes. Mechanical injury induced by pressure brings about a contraction of the protoplasm which in its early stages resembles plasmolysis and which is often reversible if the injury is slight. It passes through a plasmolysis-like stage to an irreversible granulation if the pressure is considerable or of long duration. Strong light (**13**) and high temperature likewise cause retraction of the protoplasm from the cell walls, and light and temperature conditions which are not markedly injurious may influence the time required for plasmolysis.

In the second place, the assumption made by URSPRUNG and BLUM (**9**), and with few exceptions accepted without experimental proof by subsequent workers, that the cell contents and applied solution come into equilibrium within 20–30 minutes, can be shown to be untenable both on theoretical

[1] This work was done at the Imperial College of Science and Technology, London. The writer wishes to express her thanks to Professor V. H. BLACKMAN for his interest and advice.

grounds and on the basis of experiments such as those to be presented here. An uninjured protoplast, when immersed in a hypertonic solution, will lose water to the solution at a rate proportional to the difference between the osmotic pressure of the external solution and that of the cell contents. The rate of water loss from the cell will decrease as the internal osmotic pressure, owing to concentration of the sap by loss of water, increases to a value nearer that of the outer solution. A protoplast, immersed in any given solution and showing at any arbitrarily chosen moment incipient plasmolysis, may therefore subsequently contract further and may even reach the completely "balled" stage if observed for a long enough time. *A persistent incipient plasmolysis is an unattainable state.* The factors which Höfler (**7**, p. 301) enumerates as determining the decrease in size of a protoplast in a given solution, namely, conditions of approach of the solution to the cell, permeability of the cell wall to the dissolved substance in the plasmolyticum, passage of water through the plasma from the cell sap, etc., may also affect to some extent the plasmolysis time (*i.e.*, the time before the first appearance of plasmolysis) of comparable cells in different solutions. Persistent plasmolysis is not only unattainable, but it would also appear useless to assign an arbitrary time limit after which approximate equilibrium between the cells and the plasmolyticum should be assumed, and meaningless to give plasmolysis results in terms of both concentration and time.

In the present work an attempt has been made to rule out the first objection, and to evaluate the significance of the second. Sections were replaced by "strip" preparations, as discussed elsewhere (**3**). Cells which were uninjured and which had not been in contact with injured cells were obtained in the following manner. A sharp scalpel was inserted obliquely into the surface tissues of the leaf or other organ to be studied and the epidermis torn away. This was in many cases accompanied by a single stratum of mesophyll cells which had been subjected to no injurious influences other than the slight strain involved in separating them from the subjacent tissues. Only cells situated at least three cells distant from the periphery of the preparations were examined, since the behavior of cells at the periphery is always anomalous. Strips prepared in this way were cut into convenient lengths, immersed in sugar solutions of graded concentration, and the time elapsing between immersion and the first appearance of plasmolysis recorded.

The validity of the plasmolytic method as a measure of the osmotic value of the cell sap, assuming that uninjured cells have been used and mechanical and pseudo-plasmolytic factors are ruled out, rests on the assumption that a relation exists between concentration of plasmolyticum and time required for completion of plasmolysis such that a definite concentration can be determined above which plasmolysis occurs within a brief

time while below this concentration plasmolysis will not occur even after a long period. Such a condition is illustrated in the ideal curve of figure 1, *A*. Here plasmolysis would not occur after any length of time in a plasmolyticum of 0.450 M concentrations, while at all concentrations above 0.470 M plasmolysis would be evident within 20 minutes after immersion. Figures 1, *B* and 2 illustrate the experimental data for the seven plants tested. The ideal curve is rather closely approximated by the experimental

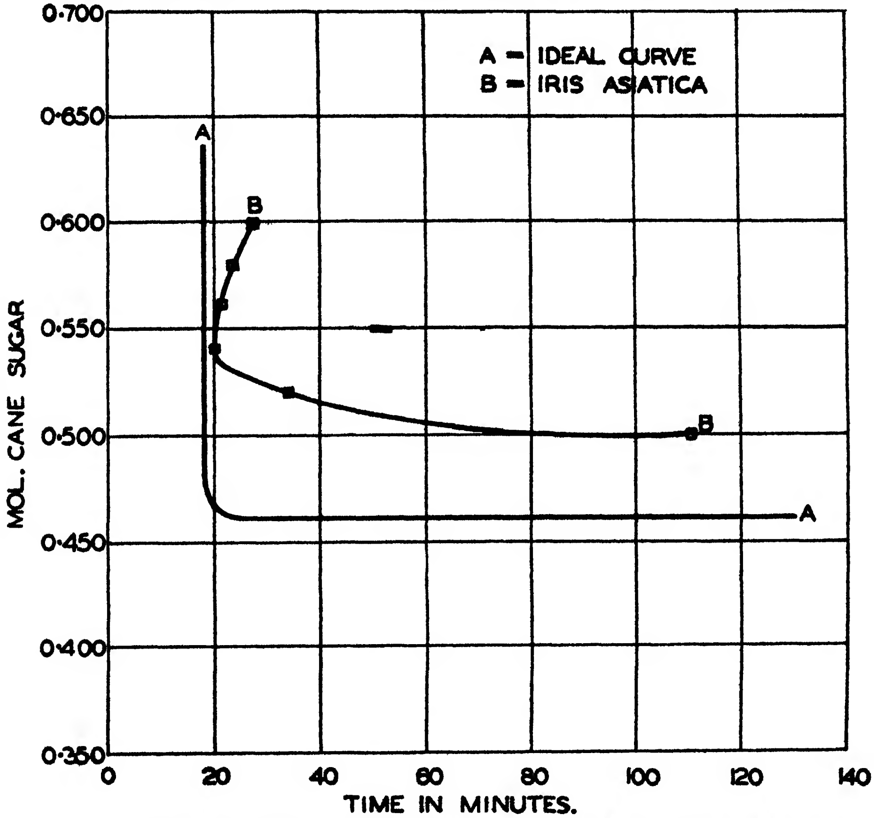

FIG. 1. Time of immersion in different concentrations of cane sugar before observed inception of plasmolysis: *A*, ideal curve for determination of osmotic pressure by the plasmolytic method; *B*, observed curve for stripped preparation of *Iris asiatica*.

curve for *Iris asiatica* (fig. 1, *B*). Here the critical concentration lies somewhere between 0.500 and 0.530 M, since at 0.500 it required two hours for plasmolysis to appear while at 0.530 M and all higher concentrations a similar state was reached within 20–30 minutes. The slope of the curve above $C = 0.530$ was thus ∞, while below that value it was 0 (approximate). If such were always the case, plasmolysis would be a satisfactory means of measuring osmotic value. This, however, is not the case. In the curve

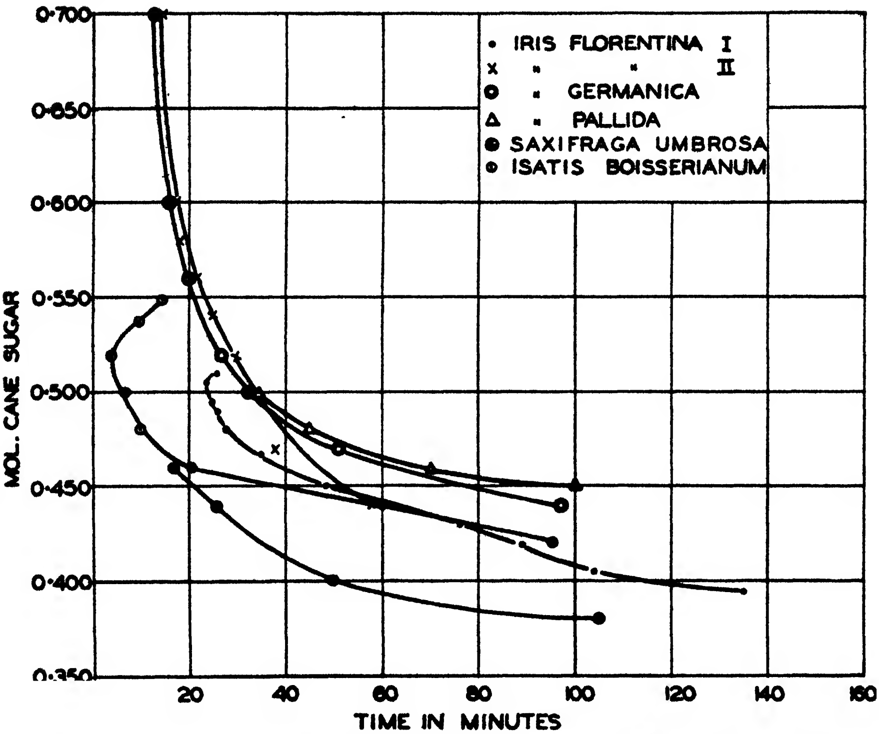

FIG. 2. Observed concentration-time curves for inception of plasmolysis in stripped preparations of several plants; compare with ideal curve of figure 1, *A*.

obtained for *Iris germanica* (fig. 2), the value $\frac{\Delta C}{\Delta T} = \infty$ is reached only above $C = 0.600$, while $\frac{\Delta C}{\Delta T} = 0$ is reached only below 0.425. At about $C = 0.480$ the slope of the curve is 1 and deviations from that value in both directions are slow. There is no *critical* point. The other experimental curves are similar. Of the seven plants studied *Iris asiatica* is the only one which gave data showing any semblance of a critical point. Since the whole method rests on the assumption of the existence of such a point, its invalidity is obvious.

The plasmolytic method as formerly used is therefore invalid because it does not deal with tissues in normal, healthy condition, because it frequently does not differentiate between true osmotic phenomena and pseudo-plasmolytic mechanical phenomena, and because when these factors are eliminated and the phenomenon considered as a concentration-time relationship it gives critical values in only rare instances. It seems very doubtful whether it will ever be possible to eliminate all of these objections satisfactorily.

A curious phenomenon, a slower appearance of plasmolysis in high concentrations than in low ones, is illustrated in figure 1 (*Isatis boisserianum* and *Iris florentina*). This anomaly was observed in a number of experiments, and in every case the cells appeared healthy, and except for the time relation plasmolysis appeared to follow the normal course. The explanation is not clear, but temperature seems to play some part in the process, as the delay of plasmolysis in high concentrations did not occur in tests made somewhat late in the day when the room temperature was higher, or on warm days.

In yet higher concentrations the cells sometimes show internal plasmolysis and sometimes fail entirely to plasmolyze, while still appearing perfectly healthy. This failure to plasmolyze is not a case of deplasmolysis which has been overlooked. Cane sugar does enter these cells but only after some 36 hours, when its entry is probably conditioned by pathological change in the cell membrane.

Summary

The relationship between concentration of plasmolyticum and time required for plasmolysis is considered from a theoretical standpoint and the conclusion reached that considerable time is necessary before equilibrium is attained between a cell and a surrounding fluid of only slightly greater or slightly less osmotic strength. Data on this relationship are presented. When mechanical injury and the pathological actions of fluids from ruptured cells are eliminated, and when temperature and light are those of normal laboratory conditions, plasmolysis is a slow process; and limiting plasmolysis is so much deferred that secondary changes must be expected to have occurred in the cells such as would render invalid the interpretation of plasmolysis data as any measure of the osmotic value of the contents of plant cells.

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PANTOTHENIC ACID AS A NUTRILITE FOR GREEN PLANTS

ROGER J. WILLIAMS AND EWALD ROHRMAN

Introduction

Ever since the work of BOTTOMLEY on the stimulating effect of extracts of bacterized peat on the growth of green plants, there has been some interest in the possibility that green plants may require an outside source of specific organic material. The preponderance of opinion (**1, 2, 6**) seems to favor the idea that, while stimulating effects may exist, the green plant is able to grow without any such outside stimulus and hence is independent in accordance with the classical view.

It has been suggested (**6**) that the term "auximone," coined by BOTTOMLEY, should be dropped from the literature because the substances are not indispensable and the experimental data from which their existence was inferred were based upon an unfavorable salt balance. It is claimed that auximones, as conceived by BOTTOMLEY, do not really exist. In this connection we again call attention to the advantage in the term *nutrilite* (**3**), which carries no presuppositions except that the vitamin-like substance functions in nutrition.

Up to the present, so far as we are aware, no substantial clue which will stand scrutiny has been obtained as to the chemical nature of the substances which are presumed to exist and to stimulate the growth of green plants.

Experimental work

The first suggestion that pantothenic acid (**4**) might be involved in this problem came from a study of the pantothenic acid content of dormant and sprouting potatoes. It was found that while the pantothenic acid is rather uniformly distributed throughout the tissue of dormant potatoes, during sprouting there is a distinct mobilization of the acid toward the sprouting end. This suggested that it might have a growth stimulating function as is the case with yeast.

Preliminary work, conducted about the same time by Dr. W. L. POWERS of the Department of Soils, showed that one of our relatively crude concentrates had a stimulating effect on alfalfa seedlings. The details of these and other tests will be published elsewhere.

Through the initiative of the junior writer, experiments were conducted with an aquatic plant which, so far as we know, has not been used for physiological study. Young plants of *Ricciocarpus natans* were collected from an aquarium and later from a marshy lake, and cultured on

Detmer's solution. The first effects were observed visually. When the young plants grow the two lobes separate as a new lobe grows between them. It was noted that this evidence of growth was much more apparent in cultures to which from 10 to 10,000 parts per billion of a crude pantothenic acid concentrate (calcium salt potency of 70) was added. The best result was obtained with 1 part per million and the difference became definitely noticeable on the third day. In another experiment there was visual evidence (recorded after 12 days) of improved growth when as little as 1 part per billion of a refined preparation (potency 5000) was added. When 10 and 100 parts per billion were added the growths were better than the control but not so good as when only 1 part per billion was present.

In a third experiment, two carefully selected plants of uniform size were grown in each of four dishes. Those to which additions of the refined preparation just described were made showed some increased growth in 5 days; and after 9 days the plants were blotted uniformly between filter papers and weighed in a closed vessel on a micro balance, with the results indicated in table I.

TABLE I

EFFECT OF PANTOTHENIC ACID ON GROWTH OF *RICCIOCARPUS NATANS*

	WEIGHT OF PLANTS
Blank	0.002545
1 p. p. billion	0.003210
10 p. p. billion	0.004350
100 p. p. billion	0.003840

It is evident that 100 parts per billion is an overdose for this particular preparation. It should be noted also that while the medium used may not be ideal for the liverwort, the amount of material added is extremely minute, and its effect must therefore be specific.

Further experiments indicated that the liverwort used in these experiments is not a very favorable plant for continuous laboratory culture, because the young plants are available only after sporulation and this event cannot be controlled in the laboratory. The physiological condition of the plants is an important factor. The plants which showed the most striking responses were those obtained from the aquarium. Those taken from the natural habitat were less responsive, and after keeping for a few weeks the plants in one case were apparently unaffected by the more refined preparation, except that 100 parts per billion actually inhibited growth.

Within a few days after these experiments had been completed, a communication was received from Professor W. J. ROBBINS indicating that in preliminary experiments he had obtained positive growth stimulating results with a pantothenic acid preparation which we had furnished. His study utilizing isolated root tips from corn will doubtless be published shortly.

Discussion

These studies with four different plants (including the potato), even though they are not exhaustive, would seem to indicate that pantothenic acid plays a rôle in the growth of green plants generally. This idea is strengthened by the evidence previously presented (**4**), that pantothenic acid is a constituent of all types of living matter.

To the plant physiologist mainly will fall the problem, which will probably prove an intricate one, of finding precisely how pantothenic acid functions. The writers cannot answer this question and available facilities will probably severely limit our further work along this line. We wish, however, to mention a few observations which are an outgrowth of our limited experience with green plants and our more extended acquanitance with the chemical factors influencing yeast growth.

ORIGIN OF PANTOTHENIC ACID.—The observation has been recorded elsewhere (**4**) that *Aspergillus niger* produces pantothenic acid as it grows upon a synthetic medium. We have also observed that several bacteria and other molds which can grow upon synthetic media also produce it. It is not surprising, therefore, that the substance is present in soils and especially abundant in those soils which are rich in organic matter. Our experience would lead us to suspect that it is produced neither by higher plants nor by animals. We have conducted careful experiments to prove whether or not it is produced as yeast grows (extremely slowly) in a synthetic medium or one with known low pantothenic acid content. The results indicate that it is not produced. Yeast grown on a medium low in pantothenic acid content contains very little pantothenic acid, as low as one-fiftieth as much as "normal" yeast and even less than that introduced originally into the medium. The medium at the end of the growth period was practically devoid of pantothenic acid. It seems that under these conditions the pantothenic acid present in the mother cells is distributed to the daughter cells, and that growth reaches practically a standstill in the course of a few days, owing to its very low concentration.

IS PANTOTHENIC ACID INDISPENSABLE OR MERELY STIMULATIVE?—In the light of the experiments just cited, one can draw his own conclusions as to the indispensability of pantothenic acid for yeast growth. If the conclusion is that the effect is that of a stimulant, the interest in the stimulating

substance should not be diminished, because no one can question but that under anything approaching natural conditions yeast is always stimulated by pantothenic acid, whether or not it is capable of growing very slowly without that stimulation.

To us the possibility that pantothenic acid is stimulative rather than indispensable to green plants should not lessen interest in it. Under natural conditions plants are doubtless stimulated by the substance because it is found in soils generally and the organisms of the soil are capable of producing more when the available supply runs low, of course, provided that the soil is suitable for the growth of these organisms. Just how long a plant can grow and eke out an existence without a further supply of organic matter beyond that supplied by the seed is not very important, if under ordinary circumstances it obtains specific organic matter from outside sources and grows normally because of this exogenous supply.

Are other nutrilites involved in green plant stimulation besides pantothenic acid?—Our experimental evidence with regard to this point is scanty, yet we have observed in work with the liverworts that an extract of potato seemed to have a stimulating effect well beyond that which could be accounted for by its pantothenic acid content alone. Since the physiological condition of the test organism could not be standardized, the results were not of a quantitative nature, and a definite conclusion would therefore be dangerous.

From our work with yeasts we should not be surprised if factors other than pantothenic acid were involved. Not all of the various strains of *Saccharomyces cerevisiae* are stimulated markedly by pantothenic acid alone. Other complementary factors may be necessary to produce even approximately normal growth. In the case of two yeasts, crystalline vitamin B_1 fills this need. What the nature of the complementary substance is in the case of green plants we do not know, but we expect to be able to answer the question as to their existence and nature by further experimentation. From analogy with yeast growth experience, we should expect that plant growth stimulation could be induced much more markedly than has been possible if all the unknown factors were supplied to the growing plant.

Effect of toxic factors.—Not only with yeast but in our limited experiments with green plants we have observed the influence of inhibiting substances. In yeast growth experiments we are constantly confronted with such effects. We have natural extracts which are devoid of any ability to stimulate yeast growth because they contain toxic material which is specific for a given yeast. By fractional electrolysis (5) we are able to prepare very active stimulating material from the same extract. Our experiments indicate that such results will carry over to experiments with green plants and will significantly modify results. There is also the possibility

that an unfavorable balance between various complementary growth-stimulating substances may result in growth inhibition. This appears to take place with yeast in some cases.

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Summary

1. Pantothenic acid has been found in preliminary experiments to have a growth stimulating effect on green plants.

2. The questions of whether it is stimulative or indispensable, where it originates in nature, and the probability of the existence of other plant nutrilites, are discussed.

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SOLUBILITY OF POTASSIUM IN CORN TISSUES[1]

V. H. MORRIS AND J. D. SAYRE

Introduction

In a recent paper (6) it was suggested that the composition of succulent plant tissues with respect to any constituent in true solution in the cell sap can be calculated from an analysis of sap expressed from the tissues. In applying this method of analysis to studies of the mineral nutrition of the corn plant, it was necessary to ascertain whether the potassium was entirely in solution in the cell sap. If this situation obtained, the method of analysis could be used to determine the total potassium content,—in some investigations a much simpler and more convenient procedure than the usual ash analysis involving drying and grinding the sample.

In investigating the potassium content of expressed corn sap, data were obtained which are believed to furnish an answer to the question as to whether potassium occurs in corn tissues entirely in expressible form or whether it may be partially fixed by association with cell constituents such as lignin. The data are presented in this paper.

The literature bearing on this subject is generally interpreted as leading to the conclusion that potassium occurs only in solution in the cell sap, and probably in inorganic form (5, 1). Recently, however, McGEORGE (4) presented evidence suggesting the possibility of a system of base exchange in living plant tissues similar to that already demonstrated for soils, where a correlation has been found between such factors as lignin content of the organic matter and exchange capacity. INOZEMTZEV (3) has concluded from electrodialysis studies that the greater part of the potassium of plants exists in a complex nondialyzable form, and that the dialyzable potassium is probably in an organic form.

Material and methods

The material consisted of samples taken during a seasonal study of the distribution of potassium in the different parts of the corn plant. A single cross adapted to Wooster conditions was grown on a fairly uniform soil in a good state of fertility. On each sampling date several plants were brought into the laboratory and separated into the following parts: blade, sheath, upper stem (above ear), lower stem, tassel, husks, grain, and cob. Each part was finely cut in an electric foodchopper. The potassium content was calculated from an analysis of the sap expressed from portions of the ground tissue, as previously described (6). Samples of the ground tissues also were

[1] Investigations cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry and Department of Agronomy, Ohio Agricultural Experiment Station.

dried in a steam chamber and then prepared for analysis. Portions of a few of the samples also were extracted with hot water or with 5 per cent. alcohol, using 50 gm. of the green tissue in Soxhlet extractors and making the extract to a final volume of 500 ml.

Potassium was determined in the various kinds of samples after ignition with sulphuric acid, using 10-ml. aliquots of the expressed sap, 2.5 gm. of dried tissue, and 100 ml. of the extracts. The ashed samples were taken up in hot dilute HCl, filtered, and washed into 250-ml. volumetric flasks. The potassium was precipitated from 50-ml. aliquots by the chloroplatinate method (**2**), first removing the calcium as the oxalate. All samples were taken in duplicate and each value reported is the mean of the duplicates.

Results

It was desirable to determine first whether there was any potassium which was in an insoluble form or held by adsorption and that could be obtained by continued extraction but not by the sap expression method. The results obtained with a series of samples, reported in table I, do not indicate the

TABLE I

POTASSIUM CONTENT OF CORN TISSUE AS DETERMINED BY SAP EXPRESSION, EXTRACTION, AND ASH ANALYSIS OF DRIED TISSUE (PERCENTAGES ON GREEN WEIGHT BASIS)

DATE	TISSUE	SAP EXPRESSION	EXTRACTION		TOTAL IN DRIED TISSUE
			H_2O	5% ALCOHOL	
		%	%	%	%
June 30	Whole plant	0.23	0.25	0.25	0.27
July 7	Blade	0.35	0.36	0.30	0.34
July 14	Blade	0.43	0.45	0.35	0.46
	Stem	0.18	0.19	0.19	0.18
July 21	Blade	0.36	0.38		0.40
	Sheath	0.26	0.26	. .	0.25
	Upper stem	0.18	0.13	. . .	0.18
	Lower stem	0.13	0.11	..	0.15
July 28	Blade	0.43	0.45	.. ..	0.49
	Lower stem	0.17	0.13	.. ...	0.17
	Ear	0.20	0.15	. .. ..	0.20

presence of potassium which could be extracted but not expressed. Furthermore, as shown in the same table, comparison with the analysis of dried tissue shows that all of the potassium was obtained by both extraction and sap expression.

Further evidence that all the potassium is in solution in the cell sap and consequently can be determined in corn tissue by the sap expression method,

as well as by analysis of dried tissue, is afforded by the data from a series of 56 samples, the results of which are summarized in table II. Although the

TABLE II

POTASSIUM CONTENT OF CORN TISSUES AS DETERMINED BY SAP EXPRESSION AND ASH ANALYSIS OF DRIED TISSUE (PERCENTAGES ON GREEN WEIGHT BASIS)

TISSUE	NUMBER OF SAMPLES AVERAGED	SAP EXPRESSION	TOTAL IN DRIED TISSUE
		%	%
Blade	10	0.389	0.414
Sheath	7	0.319	0.317
Upper stem	7	0.176	0.189
Lower stem	9	0.184	0.178
Husks and silks	4	0.130	0.157
Grain	3	0.190	0.180
Cob	5	0.152	0.192
Hybrids (stem tissue)	11	0.165	0.185
Mean of all samples		0.226	0.240

mean of the 56 samples is slightly higher for total potassium in dried tissue, the difference (0.014) is not significant, since the standard deviation of the difference between the two methods is 0.029.

The only samples with differences large enough to approach significance were the last two samples of cobs, taken on August 18 and 25. The potassium contents by the sap expression method were 0.128 and 0.143 respectively, as compared with 0.204 and 0.247 for dried tissues. Whether these differences are due to discrepancies in technique, to mechanical difficulties in handling this kind of tissue, or actually represent the presence of potassium held in such a way that it could not be expressed, cannot be determined from these data. That it may well be due to the mechanical difficulty of expressing sap from cobs rather than to the high percentage of lignin in these tissues is indicated by the fact that the first three samples of cobs showed good agreement between the methods.

With the exception noted, the data indicate that the potassium in corn tissues is in solution in the cell sap and thus can be determined by analysis of expressed sap as well as by analysis of dried tissue. Since all the samples were first ignited with sulphuric acid, however, it is possible that some of the potassium might be in solution in organic form, or at least unionizable. Data obtained by the direct precipitation of the potassium in a cleared portion of the sap by sodium cobaltinitrite are presented in table III. The results by the different methods are in good agreement. From these it appears that all the potassium in corn tissues is in such a form that it can be directly precipitated by regents such as sodium cobaltinitrite, indicating that it is in true solution and ionizable.

TABLE III

POTASSIUM CONTENT OF CORN TISSUE DETERMINED BY DIRECT PRECIPITATION FROM EXPRESSED SAP AS COBALTINITRITE COMPARED WITH ASHED EXPRESSED SAP AND DRIED TISSUE ANALYSIS

TISSUE	TOTAL IN DRIED TISSUE	SAP EXPRESSION	
		GRAVIMETRIC	COLORIMETRIC
	%	%	%
Blade	0.40	0.36	0.36
Sheath	0.25	0.25	0.26
Upper stem	0.18	0.18	0.19
Lower stem	0.12	0.13	0.14
Tassel	0.31	0.27	0.28

The assumption that a base exchange system exists in the plant implies, by analogy with the well known soil system, a tying up of bases, potassium for example, in insoluble form in absorption complex or chemical combination. From the data presented in this paper it may be concluded that with respect to potassium such a system does not obtain in the corn plant.

Summary

1. The potassium in corn tissue is entirely in solution in the cell sap and consequently can be determined by analysis of expressed sap as well as by extraction or dried tissue analysis.

2. With the possible exception of cob tissue, there is no evidence of the presence in corn tissues of any insoluble, fixed, or unionizable forms of potassium.

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PHOTOELECTRIC APPARATUS FOR MEASURING LEAF AREAS[1]

DONALD E. H. FREAR

(WITH THREE FIGURES)

The need to measure accurately the area of several thousand apple leaves in connection with a study of the deposit of spray residues led to a search of existing methods of leaf area measurement. The several methods described in the literature failed to satisfy the requirements, inasmuch as accuracy in area measurements was apparently to be secured only by methods too lengthy to be of use in this study.

Existing methods for measuring leaf areas include the following: (1) Measurement of the leaves along their axes, the area obtained by multiplying these figures being corrected by a factor; (2) planimeter measurements; (3) tracing or otherwise transferring the leaf outline to paper of known weight per unit of area and later determining the area of the tracings by their weight; (4) leaf-punch methods, in which definite areas of the leaf surface are removed by a punch or die.

The first of these methods is not sufficiently accurate, since variations in leaf shape among individual leaves of the same species is sufficient to introduce gross errors into the calculation. The second and third methods are extremely time-consuming, depend largely for their accuracy on the skill and dexterity of the person using them, and are adapted only for measuring the areas of small numbers of leaves. Circles or other geometric figures cut from the leaf surface with a punch or die are subject to errors arising from the obvious fact that the leaf presents neither a uniform surface nor an interior structure free from gross tissue differentiation.

The use of the photoelectric cell in a variety of ways in recent years led the writer to consider the possibility of such a device for measuring leaf areas, inasmuch as the area would be a direct function of the amount of light intercepted if the leaf were placed in the path of a beam of light. While a device was being perfected, a short news article appeared describing a similar apparatus, built by WITHROW, and it was learned that a paper describing a similar apparatus had been presented at the Boston meeting of the American Society of Plant Physiologists. Definite information concerning the construction of this apparatus could not be secured, however, and the present apparatus has been developed independently, and has been used satisfactorily for the past season.

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, as Technical Paper no. 677.

Apparatus

A rectangular box 72 cm. high, 22 cm. deep, and 22 cm. wide was constructed from plyboard, with a tight fitting door at the front. The interior of the box was painted white and near the top a number of holes were bored for ventilation. A perpendicular section through the apparatus is shown in figure 1. A movable shelf *A*, to which were attached five 60-watt 110-

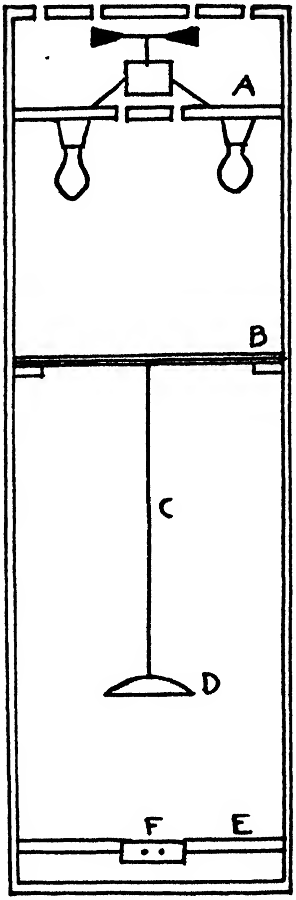

Fig. 1. Section through apparatus.

volt inside frosted electric lamps, was inserted near the top of the box. These lamps may be connected to a constant wattage transformer so that current variations may be reduced to a minimum. On this shelf was placed a small electric fan to circulate the air. Across the middle of the box was placed a pane of frosted glass, of such size that it exactly fitted the interior of the box, but masked so that only a circular opening approxi-

mately 18 cm. in diameter was available to transmit light (fig. 1 *B*). A small hole was drilled in the exact center of the circle of frosted glass so circumscribed, and a hemispherical baffle *D* was suspended by a glass rod *C*, approximately 15 cm. below the frosted glass plate. A sheet of heavy plate glass of the same size as the frosted glass was laid on top of the latter.

At the bottom of the box, about 4 cm. above the true bottom, a false bottom *E* was built, in the center of which a hole large enough to accommodate the photoelectric cell was cut. This photoelectric cell, a Weston Model 594, was connected with a microammeter having a capacity of 200 microamperes.

The purpose of the hemispherical baffle is to deflect all light rays passing through the frosted glass which would otherwise fall directly upon the surface of the photronic cell. This makes it possible for the cell to measure the *diffused* light in the lower chamber, rather than to give erratic readings because of differences in the location of shaded areas. Such differences would exist if the object causing the shading (in this case the leaves) were located in different places on the ground glass.

The intensity of the illumination in the lower chamber may be regulated by raising or lowering the bank of lamps at the top of the box.

Standardization

In standardizing the instrument, the light bulbs are connected to a 110-volt current source and adjusted so that the reading of the microammeter connected with the photronic cell is nearly the maximum of the instrument. This original reading is recorded. A section of definite area cut from a leaf similar to those to be measured is then placed dorsal side uppermost, upon the ground glass plate, and held in a flat position by the sheet of plate glass. The reading of the microammeter is again taken and the process repeated, adding a section of leaf of known area each time until the ground glass plate is covered to its capacity. A curve is then prepared, plotting the percentage of light cut off against leaf area. The percentage of light cut off is calculated by subtracting the microammeter reading taken for any given leaf area from the original reading, and dividing this by the original reading.

Variations in the electric current cause fluctuations in the reading of the microammeter, but it has been found that over the relatively short time necessary to make the standardization or to take the readings on a series of whole leaves, as will be described later, these fluctuations are not large enough to cause serious error. The use of the constant wattage transformer mentioned reduces these fluctuations greatly. It is wise to repeat the standardization several times, however, so that each point on the curve rep-

resents the mean of several readings on the microammeter. A typical standardization curve is shown in figure 2.

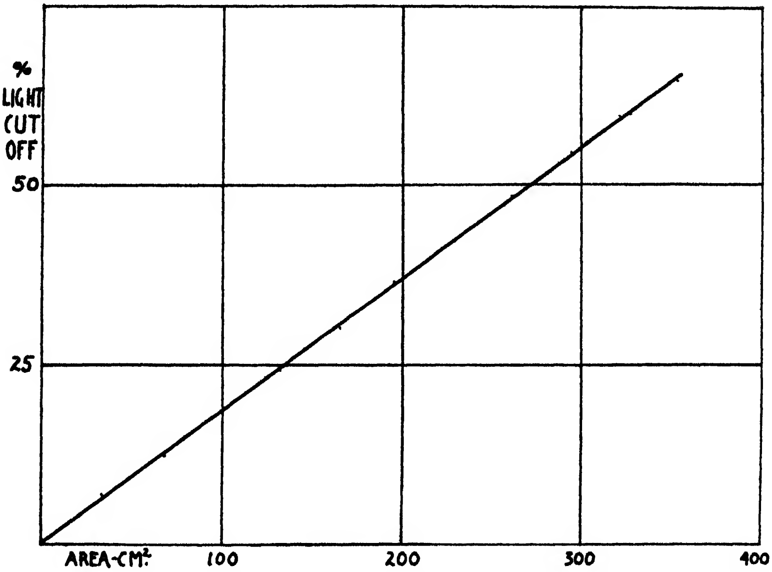

FIG. 2. Standardization curve.

Measurement of leaf areas

Having constructed a curve for the type of leaves to be measured, the actual measurement of the leaf area is carried out in a manner similar to the standardization. The original reading of the microammeter is taken without any material on the ground glass plate to interfere with the passage of the light. The leaves to be measured are then placed on the ground glass, one at a time, or, if the total area of a number of leaves is desired, several leaves at a time, and the second reading of the instrument taken. It has been found that with an apparatus of the dimensions given, four or five mature apple leaves may be placed upon the ground glass at one time, and the area of 100 leaves measured in less than 15 minutes. To guard against wide fluctuations in current, it is wise to take original readings after each five sets of leaf area readings.

The percentage of light cut off by each leaf or group of leaves is then calculated, and by reference to the standard curve, the area is determined. An example of the calculation is as follows:

Original reading . . . . 188 microamperes
Reading with 4 leaves . . . . 103 microamperes

$$\frac{(188–103)}{188} = 44.0\% \text{ of the original light cut off.}$$

Referring to the curve given in figure 2, this indicates an area of 238 square centimeters.

Discussion

In building this apparatus an attempt has been made to avoid any unsymmetrical construction which might cause shadows, or tend to give a different light intensity on the photronic cell owing to a variation of the position of the leaf or leaves to be measured when placed upon the ground glass. With the instrument here described, it makes no appreciable difference whether the leaf is in the center of the circular opening on the ground glass or far to one side.

The method of standardization of the instrument may introduce serious errors in the determination of leaf areas by any photoelectric method. Leaf tissue, except perhaps in rare cases, is appreciably translucent. In addition, the reflection of light from the surface of a leaf is characteristically different from the reflection from many other materials. In fact, the reflection of light from the upper surface of a leaf is usually different from the reflection from the lower surface. This is particularly true if the lower surface is pubescent. For these reasons, then, the standardization of the instrument against known areas of opaque material, or against material of different color or reflectivity from that of the leaf surface, may lead to inaccurate results. Figure 3 shows typical curves obtained by standardizing the instrument with known areas of black paper (*A*); white cardboard

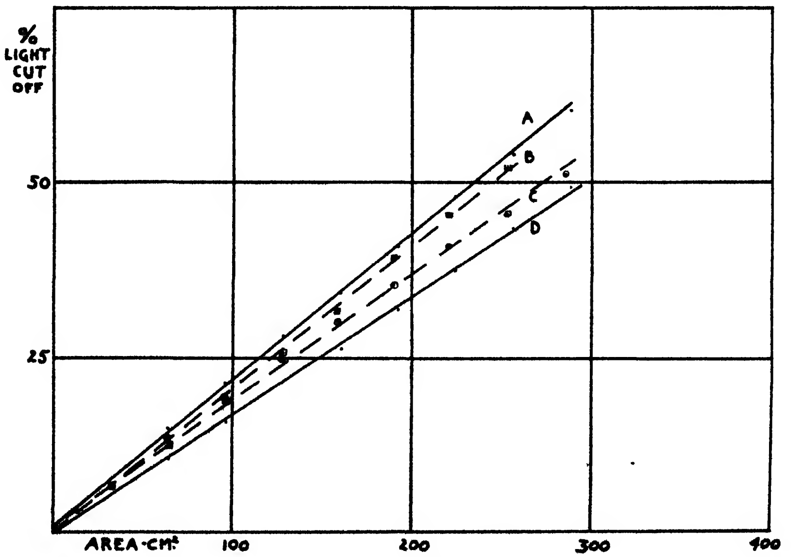

FIG. 3. Comparative standardization curves.

(*D*); and two types of leaf tissue, one thick and practically opaque (*B*) and the other relatively thin and translucent (*C*).

The use of alternating current to provide illumination admittedly increases the error of measurement. It is possible, however, through the use of a transformer to keep this at a minimum, and frequent checking of the current through microammeter readings helps reduce this error.

This apparatus has been used satisfactorily to measure the area of more than 10,000 apple leaves during the past season, with an error of approximately 3 per cent., as determined by replicate area measurements on the same leaves.

Summary

An apparatus is described for the measurement of leaf areas by means of the photoelectric cell. The chief advantages of the method are its rapidity and simplicity, with a high degree of accuracy. The methods of standardization and calculation are given in detail. The apparatus has been used to determine the area of more than 10,000 apple leaves, with an error of approximately 3 per cent.

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NOTES ON THE SUCROSE CONTENT AND DEXTROSE–LEVULOSE RATIO OF CALIFORNIA DRIED PRUNES[1]

The quality of dried prunes in California is to some extent related to the districts in which the fruit has grown. The principal variety grown in all sections is the French prune d'Agen. The four principal prune-growing districts are the Santa Clara and the Napa-Sonoma which are often designated as "inside" districts, and the Sacramento and the San Joaquin valleys which are often called "outside" districts in the prune trade. The inside districts are near the coast where the temperatures are lower. Less irrigation is necessary or customary in the coastal or inside districts, it is more common to use short pruning methods, and the crops average smaller. In the inside districts the fruit normally falls to the ground when in best condition for drying, while for some undetermined reason the fruit in the outside districts in large measure remains on the tree until deterioration is marked unless shaken or knocked to the ground.

TABLE I

TOTAL SUGAR CONTENT* OF SAMPLES OF THE 1932 CROP

	INSIDE DISTRICTS	OUTSIDE DISTRICTS
Average	49.4	40.5
Maximum	53.3	45.1
Minimum	46.4	37.3

* Expressed as invert sugar, adjusted to a basis of 20 per cent. moisture.

TABLE II

TOTAL SUGAR CONTENT* OF SAMPLES OF THE 1933 CROP

	INSIDE DISTRICTS			OUTSIDE DISTRICTS			ALL DISTRICTS
	SANTA CLARA	NAPA-SONOMA	ALL	SACRAMENTO	SAN JOAQUIN	ALL	
Average	54.7	49.5	52.1	45.1	45.8	45.5	49.4
Maximum	59.4	51.5	59.4	47.2	50.9	50.9	59.4
Minimum	47.7	47.2	47.2	42.7	42.3	42.3	42.3

* Expressed as invert sugar, adjusted to a basis of 20 per cent. moisture.

[1] This brief paper was prepared by Professor NICHOLS shortly before his untimely death in November, 1934.

TABLE III

SUCROSE CONTENT OF SAMPLES OF THE 1933 CROP

INSIDE DISTRICTS				OUTSIDE DISTRICTS			
SANTA CLARA		NAPA-SONOMA		SACRAMENTO		SAN JOAQUIN	
COUNT PER LB.	SUCROSE	COUNT PER LB.	SUCROSE	COUNT PER LB.	SUCROSE	COUNT PER LB.	SUCROSE
	%		%		%		%
74	0.95	37	10.26	39	3.23	64	0.95
44	4.37*	46	6.18	49	7.79	86	6.84
49	0.88*	61	10.35	66	8.08	120	4.18
73	5.42*	82	8.17	90	7.69	60	3.61
77	6.18*	107	8.64	109	4.18	83	5.13
122	12.05*	40	1.52	39	3.99	98	9.31
40	1.33	64	7.50	70	7.79	47	1.52
49	7.12	45	6.08	47	6.19	68	3.61
68	7.60	80	3.23	88	4.84	78	1.71
78	4.94	108	2.95	108	10.74	113	2.18
104	3.61	79	4.27				
116	1.90	68	7.22				
68	5.22	102	5.50				
93	1.16	133	2.37				
113	5.13						

Av.	4.51	Av.	6.02	Av.	6.42	Av.	3.90
Max.	12.05	Max.	10.35	Max.	10.74	Max.	9.31
Min.	0.88	Min.	1.52	Min.	1.90	Min.	0.95
Inside districts:		Average	5.24	Maximum	12.05	Minimum	0.88
Outside districts:		Average	5.06	Maximum	10.74	Minimum	0.95
All districts:		Average	5.12	Maximum	12.05	Minimum	0.88

* Samples in subgroups within columns are of different sizes from single grower's delivery.

The inside districts have the reputation of producing fruit of higher quality than that of the outside districts. It has been shown by HILTNER and HATHERELL (2) and by GALE and CRUESS (1) that the total sugar content of dried fruit from the inside districts is higher. This has been confirmed by NICHOLS and REED (5), who also reported that the texture of the flesh is more solid and the specific gravity of the whole fruit higher in the fruit from inside districts. These facts apparently account in part for the trade preference for such fruit.

It was thought that not only the total sugar content but also the sucrose content and the dextrose-levulose ratio, on which some data have been published by MRAK, SMITH, and HENRIQUES (4), might be related to the district

TABLE IV

DEXTROSE-LEVULOSE RATIO IN SAMPLES OF THE 1933 CROP

Inside districts				Outside districts			
Santa Clara		Napa-Sonoma		Sacramento		San Joaquin	
Count per lb.	D/L ratio	Count per lb.	D/L ratio	Count per lb.	D/L ratio	Count per lb.	D/L ratio
128	2.15	37	7.25	39	2.82	64	1.98
44	2.29*	46	4.10	46	2.86	86	2.25
49	2.17*	61	4.29	66	3.35	120	2.54
73	1.87*	82	4.08	90	3.07	60	2.58
77	1.76*	107	5.43	109	2.22	83	2.75
122	2.01*	40	2.57	39	2.67	98	3.60
40	1.64	45	4.28	47	3.17	47	2.77
49	2.28	64	3.86	70	2.46	68	3.15
68	2.53	80	2.52	88	2.42	78	3.22
78	1.98	108	3.09	108	2.85	113	3.59
104	2.02	79	2.65				
116	2.35	68	2.42				
68	1.68	102	2.69				
93	2.32	133	2.42				
113	1.63						
Av.	2.04	Av.	3.69	Av.	2.79	Av.	2.85
Max.	2.53	Max.	7.25	Max.	3.35	Max.	3.60
Min.	1.63	Min.	2.42	Min.	2.22	Min.	1.98
Inside districts:		Average	2.60	Maximum	7.25	Minimum	1.63
Outside districts:		Average	2.85	Maximum	3.60	Minimum	1.98
All districts:		Average	2.63	Maximum	7.25	Minimum	1.63

* Samples in subgroups within columns are of different sizes from single grower's delivery.

of origin. Determinations of sucrose and dextrose-levulose ratio, as well as specific gravity and total sugar, were therefore made on about 60 samples representing fairly well the 1933 and 1934 crops of the principal four California districts.

The total sugar content of the 1932-crop samples was determined by the SHAFFER-HARTMANN method (**6**); those of the 1933-crop samples by the volumetric permanganate method of the Association of Official Agricultural Chemists. In both cases the total sugar was expressed as invert sugar. The 1933-crop samples were examined for total reducing sugars before inversion, also for dextrose by the LOTHROP and HOLMES (**3**) method.

The possible relation between specific gravity and total sugar content was studied, and while the correlation is by no means close there is a definite

tendency for high sugar content and high specific gravity to be associated, especially in prunes from the outside districts.

The variations in total sugar content of 1932-crop samples are shown in table I.

The variations in total sugar content of 49 samples of the 1933 crop are shown in table II.

In both years the total sugar content of samples from inside districts was distinctly higher than in samples from outside districts. The maximum values for outside districts rarely exceeded the minimum for inside districts.

The data on sucrose and on dextrose-levulose ratio are given in tables III and IV.

Neither the sucrose content nor the dextrose-levulose ratio appears to have any relation to district of origin or to size. It is of interest that dextrose greatly exceeds levulose in prunes.

Grateful acknowledgment is made of the assistance of R. D. Bethel and F. Filipello in this work, and to the United Prune Growers for samples.—
Paul F. Nichols, *University of California, Berkeley, Calif.*

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ISOLATION AND DETERMINATION OF STARCH IN PLANT TISSUE

The removal of starch from plant tissues by dispersion in various reagents has often been employed in the quantitative estimation of this substance (5). Recently DENNY (1, 2) and HOPKINS (3) have reviewed those methods wherein mineral acids or salt solutions are used as the dispersing agents. We have found that hot dilute ethanol can also be used for this purpose after the plant tissue has been suitably pretreated with an acid-alcohol reagent. In the course of a microchemical study on the reserve carbohydrates of apple wood spurs, it was noticed that after the tissue had been treated with a boiling solution of 1 per cent. nitric acid in 85 per cent. ethanol the starch granules could be dispersed in boiling 20 per cent. ethanol. A procedure employing this neutral and salt free extraction has been developed. Its essential features are presented below:

0.500 to 2.500-gm. samples (depending upon the anticipated starch content) are weighed into 40-cc. alundum crucibles. The latter are placed in a Soxhlet extractor and extracted for 24 hours with a 2:1 benzene-ethanol mixture. The crucibles are then taken from the extractor and the major portion of the solvent removed by suction. The contents of a crucible are transferred to a 300-cc. flask and covered with 180 cc. of the ethanolic-nitric acid reagent prepared by diluting 10.65 cc. of concentrated nitric acid, sp. gr. 1.40, to 1 liter with 85.1 per cent. ethanol by volume. This mixture is then boiled under reflux for 30 minutes. The residue remaining in the flask is now recovered by filtration through the original alundum crucible and washed free of acid with 75–90 cc. of hot 95 per cent. ethanol. The residue is partially dried by suction and again introduced into a 300-cc. flask. 100 cc. of 20 per cent. ethanol are then added and the contents of the flask boiled under reflux for 20–25 minutes. The insoluble matter left in the flask is now removed by filtration and washed with 40–50 cc. of boiling 20 per cent. ethanol. The filtrate and washings are combined. This solution contains the starch polysaccharides present in the original tissue.

If it is desired to isolate the starch polysaccharides the 20 per cent. ethanol extract is concentrated *in vacuo* to about 25 to 30 cc. This concentrate is poured, with stirring, into 10 volumes of a 1:1 acetone-ethanol mixture, thereby precipitating the polysaccharides which are then recovered by centrifugation and filtration.

For the estimation of the starch content of the original tissue the 20 per cent. ethanol extract is evaporated on a hot plate to a small volume (10 cc.) and 100 cc. of 2.5 per cent. hydrochloric acid added. The solution is then boiled under reflux for 2.5 hours, cooled, neutralized, and made to volume.

Glucose is determined on an aliquot portion and expressed as starch in the usual manner.

It has been observed that with certain types of plant tissue, the 20 per cent. ethanol extract contains in addition to the starch polysaccharides some non-starch polysaccharides. In such a case, a fractionation of the extract by a method such as proposed by SMALL (4) is recommended before proceeding with either the isolation of the polysaccharide from the extract by precipitation with the acetone-ethanol mixture, or the hydrolysis of the extract to reducing sugars.—CARL NIEMANN, R. H. ROBERTS, AND KARL PAUL LINK, *Biochemistry Research Laboratory, Department of Agricultural Chemistry and Department of Horticulture, University of Wisconsin, Madison, Wisconsin.*

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NOTES

Annual Election.—The annual election of the American Society of Plant Physiologists held during the last quarter of the fiscal year has resulted in the election of Dr. A. E. MURNEEK, former secretary-treasurer, as president for 1935–1936. The new vice-president is Dr. D. R. HOAGLAND, University of California; and the new secretary-treasurer is Dr. W. F. LOEHWING, University of Iowa. The results of the ballotting insure a continuation of the policies and ideals of the preceding administration which have done much to strengthen the foundations of the Society and to insure its continuous progress. Newly elected officers always appreciate the spirit of cordial cooperation on the part of members. Such cooperative effort will be especially helpful in connection with the annual meetings and programs to be presented at St. Louis in December.

Minneapolis Meeting.—The summer meetings of the Society at Minneapolis and St. Paul were very successful as measured by the criteria of attendance and interest. With the exception of the vice-presidents, the officers of the Society for 1934–1935 and 1935–1936 were present. Almost 80 members and friends attended the sessions, and great interest was shown in the various papers presented. One of the best attended programs was the symposium on seed dormancy and related problems.

About 40 members were present Wednesday at the picnic dinner at the summer home of Dr. and Mrs. R. B. Harvey on the St. Croix river. This was a most delightful social occasion, no one being allowed to be either hungry or thirsty. The visitors had the unique privilege of enjoying Dr. HARVEY's fine portrait gallery of famous plant physiologists. Music and merriment added zest at the close of the picnic. The generous and cordial hospitality of the hosts was much appreciated by everyone.

St. Louis Meeting.—Preparations for the St. Louis meeting are already in progress. It is hoped that early action may be taken on all important matters concerned with accommodations and arrangements in order that appropriate announcements may be made in the October number of PLANT PHYSIOLOGY.

It would be very helpful in preparing programs if the proposed titles could be in the hands of the program committee early. Any symposia to be organized should be arranged at the earliest possible moment, so that participants may have as much time as may be needed to prepare their contributions. Undue haste, necessitated by short time, usually lowers the value of contributions. Would it be possible to select papers from among those offered, and confine programs to a smaller number of papers, and to a reason-

able length of time? Papers which could not be accommodated with time for personal presentation might be presented as mimeographed abstracts, with opportunity for interested readers to ask questions and elaboration if desired.

These suggestions may not be practicable; but it does seem that program committees should recognize the growing need for some way to relieve the current overloading of the programs. This arises out of the effort to accommodate everyone, which has always seemed to be desirably democratic. Another way out would be to practice close segregation of related material and provide for two or more simultaneous programs in adjacent or nearby rooms.

New England Section.—A very enthusiastic report of the second annual meeting of the New England section has been received from the secretary, Dr. LINUS H. JONES, Massachusetts. The meeting was held at the University of New Hampshire, Durham, May 17–18. The officers elected for the coming year are as follows: chairman, Dr. T. G. PHILLIPS, New Hampshire; vice-chairman, Dr. B. E. GILBERT, Rhode Island; secretary-treasurer, Dr. LINUS H. JONES, Massachusetts.

The meeting was attended by men from a larger number of institutions than was the first meeting in 1934. It was voted to meet at Kingston, Rhode Island next year.

In order to emphasize the value of these regional meetings, the titles of papers presented before the gathering are presented here.

Seedling culture in sand. A. A. DUNLAP, Connecticut.

Carbon dioxide in the forest. H. I. BALDWIN, Research Forest, Hillsboro, New Hampshire.

The effect of simultaneously varied radiation, nitrogen, and potassium on the growth of white pine seedlings. R. R. GAST, Harvard.

Micro determinations of nitrogen, phosphorus, and potassium in plant material. E. A. SNOW, Harvard.

Nitrogen, phosphorus, and potassium contents of foliage of fertilized northern white pine plantations. W. H. CUMMINGS, Harvard.

Plant transpiration as modified by potassium. A. G. SNOW, Yale.

The cumulative effect of 25 years of liming with calcic vs. magnesic liming materials on the mineral nutrient composition of the edible portions of plants. D. R. WILLARD, Rhode Island.

The present status of legal recognition of the potential acidity or alkalinity of fertilizers. J. B. SMITH, Rhode Island.

Testing plants with diphenylamine. L. H. JONES, Massachusetts.

Assimilation of nitrogen by the tomato plant. H. E. CLARK, Yale.

The effect of heavy fertilization with ammonia on the glutamine content of beets. H. B. VICKERY, Connecticut.

The quantities of non-glucose reducing substances in plant juices. F. S. SCHLENKER, Rhode Island.

The determination of starch in plant tissue. G. W. PUCHER, Connecticut.

The nature of winter injury in apple trees. F. H. STEINMETZ, Maine.

Growth hormones in plants. E. A. NAVEZ, Harvard.

Experiments in highbush blueberry culture. J. S. BAILEY, Massachusetts.

The physical basis of mycotrophy in *Pinus*. A. B. HATCH, Harvard.

Ethylene induced epinasty. C. G. DEUBER, Yale.

The determination and recording of light intensities. R. H. WALLACE, Connecticut.

Respiratory systems of *Lupinus albus*. F. N. CRAIG, Harvard.

Purdue Section.—The Purdue Section reports another good series of meetings during the past year. The attendance has ranged from 20 to 35, with an average of 25. Dr. J. H. MCGILLIVRAY was president of the section during 1934–1935, A. T. GUARD secretary-treasurer, and Dr. C. L. PORTER chairman of the program committee. The titles of papers and addresses presented are as follows:

October 15, 1934, Ancient cornfields of America. PAUL WEATHERWAX, Indiana University, guest speaker.

November 5, Reproduction in fungi. G. B. CUMMINS.

November 19, Special problems of fungus nutrition. R. B. BAINS.

December 3, Reproduction in flowering plants. A. T. GUARD.

December 17, Plant hormones. D. M. DOTY.

January 21, 1935, Reports of the A. A. A. S. meetings.

February 4, Relationships existing between the functions and structures of plants. E. J. KOHL.

February 18, Present status of mineral nutrition. S. F. THORNTON.

March 4, Nitrogen metabolism. H. R. KRAYBILL.

March 18, Soil types and plant growth. S. D. CONNER.

April 1, Practical applications of fermentation phenomena. P. A. TETRAULT.

April 15, Modern trends in plant physiology. R. E. GIRTON.

The meeting on April 15 closed the activities of the year, the address closing the annual dinner festivities. The officers elected for the ensuing year are J. T. SULLIVAN, president; R. B. ZUMSTEIN, secretary-treasurer; and E. C. STAIR, chairman of the program committee.

Chemical Methods Reprints.—Reprints of the supplementary report of the chemical methods committee may be obtained from the chairman of the committee, Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building,

University of Wisconsin, Madison, Wisconsin. The price authorized for these reprints is 15 cents per copy post free. Lots of 8 ordered at one time may be obtained for $1.00 post free. Laboratories with groups of students will find this club rate economical.

The original reports published some years ago can no longer be supplied in complete sets, as some of the parts are exhausted. There are still available 4 copies of the section on soluble carbohydrates, and 38 copies of the section on peptides and basic forms of nitrogen. These also are held by Dr. TOTTINGHAM, who will furnish them as long as the few remaining sections last.

Hugo de Vries.—With the death of HUGO DE VRIES at Lunteren, Holland, on May 21, 1935, biological science has lost one of its most revered and beloved leaders. Born February 16, 1848, his life spanned the entire period of modern biological thought. To the development of experimental biology he brought a rich experience in the dramatic development of biological speculative philosophy which occurred during the several decades following the publication of the Origin of Species. He was a leader in the movement from speculative to experimental work during the last quarter of the 19th century. His great achievements in plant physiology and genetics will stand as an enduring monument to his genius. To us and to future generations of biologists his life will stand as an inspiration and a challenge to emulate his example of patient industry and creative thinking. His name will always stand high among the illustrious biological philosophers of the late 19th and early 20th century period.

Friedrich August Ferdinand Christian Went.—On July 26, 1935, Professor WENT, for many years professor of Botany at the University of Utrecht, passed away. He was elected president of the Sixth International Botanical Congress which is to convene at Amsterdam early in September, and will be greatly missed during the deliberations of the congress. He has been a great leader in the study of plant responses, and especially in the field of hormones. He was a corresponding member of the American Society of Plant Physiologists. His passing will be deeply mourned by all plant physiologists.

Annual Review of Biochemistry.—Volume IV of the Annual Review of Biochemistry has just been issued by the Stanford University Press. It maintains the high standards set by the preceding volumes, and does great credit to the editors of the series, to the reviewers, and to the publishers. In the few years since this annual review was started, it has become an indispensable guide to the rapid developments in this expanding field of research.

The current volume contains 27 reviews, a number of which will be especially useful to plant physiologists. Among these are mentioned the sections on permeability, biological oxidations and reductions, enzymes, plant pigments, the alkaloids, mineral nutrition of plants, growth substances in plants, chemistry of bacteria, etc.

The reviewers have a very difficult task to perform, since they must choose those papers for review that in their judgment contribute most seriously to the advancement in the general field covered. The sifting and winnowing of the far flung literature that is produced each year is no mean task in any field; and the provision of a reliable guide to the currents of advancement requires clear vision and sound judgment. The writer of this note feels that the great majority of the reviewers have done unusually well with their assignments.

The series is issued in handsome binding, and the press work is uniformly good. The price of volume IV is the same as for the preceding annual volumes, $5.00 per copy. The series constitutes a valuable history of the development of biochemistry in our times. Orders for this or any of the earlier volumes may be sent to the Stanford University Press.

Temperature and Living Matter.—The eighth volume of the Protoplasma-Monographien published by Gebrüder Borntraeger, Berlin, is entitled Temperature and Living Matter. The author is JAN BĚLEHRÁDEK, Professor of General Biogology, Caroline University, Prague. It is written in English, and will therefore be more readily available to American students. It contains eleven chapters with titles as follows: general principles of biological temperature action; rate of biological processes at biokinetic temperatures; variation of temperature coefficients with external and internal factors; theories of temperature coefficients; chemical properties of living systems at biokinetic temperatures; variations of morphological equilibria at biokinetic temperatures; physical properties of living systems at biokinetic temperatures; freezing and frost resistance; chilling, chill coma, and death by chilling; injury by heat and heat resistance; and stimulative effects of temperature.

There are 229 pages of text, 37 pages of bibliography and author index, and 11 pages of subject index. The work is written in simple, direct style that will be appreciated by busy readers. The ground is well enough covered on the animal side, not quite so well on the plant side. It is frequently the case that general biology is mainly animal biology in general terms. It presents a good summary of the general effects of temperature on living protoplasm.

The price quoted for this volume, RM 18, is for cloth binding uniform to the series.

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THE COURSE OF STONE CELL FORMATION IN PEAR FRUITS

WILLIAM W. SMITH

(WITH EIGHT FIGURES)

Introduction

CRIST and BATJER'S (7) analyses of the isolated grit cell clusters of pear fruits show these structures to be approximately three-fourths lignocellulose, of which about a third is lignin. This quantitative relationship was consistent enough in the several samples tested to justify quantitative estimates of the grit cell content of the fruits by lignocellulose determinations. Their histological studies revealed cell wall thickenings 20 days after blossom fall, and chemical analyses indicated lignification occurring two or three days after blossoming.

The accumulation of lignocellulose as a percentage of the dry weight proceeded rapidly for about four weeks, reaching a concentration of half the fruit's dry weight, then began sharply to decrease until at harvest time it amounted to about a fifth of the dry weight. During the period of lignification there occurred a steady decline in the relative amounts of alcohol extractable material, which reached a minimum at the time the lignocellulose reached a maximum. At this point, indicated by a decrease in the percentage of lignified tissue, began the accumulation of alcohol extractable material which continued for the rest of the growing season.

How perfectly the relative amount of alcohol extractable substances can be a reciprocal of the amounts of lignocellulose, in the two varieties of pears studied, appears in figure 21 of CRIST and BATJER'S report. For example, the Kieffer fruits on May 18 had an alcohol extractable content of 55 per cent. of the dry weight, which dropped to nearly 25 per cent. in the latter part of June, and then increased steadily, reaching a concentration of 55 per cent. again by September 2. The alcohol extract is composed largely of sugars. The lignocellulose started with a concentration of about 25 per

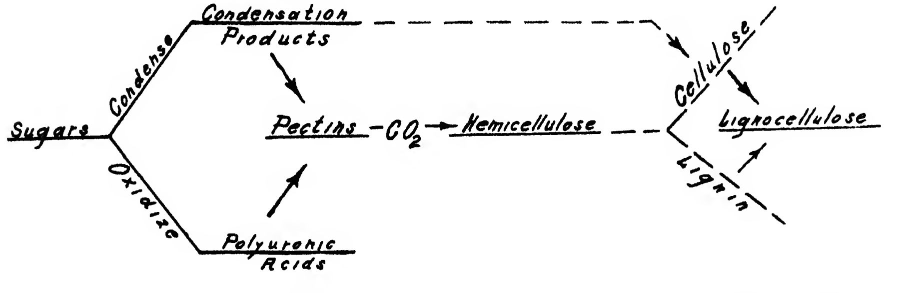
cent., reached a maximum the last of June of over 55 per cent. and then dropped to 25 per cent. by September.

This same interrelation of these materials was found in the Bartlett pear, which in comparison with the Kieffer is less "gritty" and shows a lower percentage of lignocellulose, coupled with a higher percentage of alcohol extractable substances.

It is also interesting that the "conversion point," so to speak, in this variety is about a week earlier, at which time the relative amounts of these materials correspond closely with those of the Kieffer. The regularity of this typical change in relative concentrations for all varieties of pear fruits studied, grown under different cultural conditions, in widely separated localities, for several unlike seasons, establishes it as a fundamental basic phenomenon and as the inherent order of these changes in growing pear fruits.

In general, the chemical changes incident to cell wall formation are in the direction of lignification. So far as the author is aware, the literature presents no clear cut evidence of a reversal in this order. The suggestion of CRIST and BATJER, however, that such a reversion occurs incident to the development of grit cells in the pear, lignocellulose being transformed to sugars, makes desirable a further study of the chemical changes occurring in these structures.

The results of studies of several investigators suggest a theoretical course of events in the process of lignification of plant tissues which may be indicated by the following diagram:



It is known that the first products of photosynthesis, translocated as monosaccharides or disaccharides, are sugars (**9, 21, 29**) and that the end products in the lignification of plant tissues are lignocelluloses (**11, 16, 17**). The course the sugars follow and the changes they undergo to reach this final stage is a point of fundamental concern. Many investigators have sought, chiefly on woody material, a clue to this process (**3, 5, 8, 10, 11, 21**). That the sugars condense to form polysaccharides, there is no doubt (**25, 27**). That they oxidize to form sugar acids known as polyuronic acids is supported by the results of certain research (**25, 27**).

The polyuronic acids, galacturonic, glucuronic, and others, are of particular interest because they combine with certain condensation products (arabinose, galactose) (**12, 13, 15**) to form pectins, and also, they seem to be a part of the hemicelluloses (**5, 24**).

Change of pectin to hemicellulose was fairly well established by CANDLIN and SCHRYVER (**5**). In their investigations on chemical changes taking place in cell wall substances during lignification, they group the substances accompanying cellulose in cell walls into three classes: pectins, hemicelluloses, and lignins. They were able to decarboxylate pectins with the formation of hemicelluloses which resembled in all respects the hemicelluloses isolated directly from timbers. Their results indicate that decarboxylation takes place when plant tissues lignify. They were unable, however, to establish a direct connection between pectins and lignins.

The aim of this investigation was to seek additional evidence of movement to right as indicated by the foregoing diagram (*i.e.*, from sugars through pectins, etc., to lignocellulose), to examine the possibility of a reversal of the direction of movement, and also to further the objective of relieving pear fruits generally, those of the Kieffer in particular, from the burden of grittiness in quality.

Technical methods

Sampling.—The material used for lignocellulose determinations was sampled as described by CRIST and BATJER (**7**); that is, transverse segments were cut from the center of each fruit, the loculi of the carpels removed, and the segments dried in an electric oven at 65° C. For carbohydrate and pectin analyses similar portions of the fruits were taken. These were finely ground in a meat grinder and thoroughly mixed. Small amounts were placed in weighing bottles for dry weight determinations, made at 95° C. Samples consisting of 25 grams of this material were quickly weighed and dropped into mason jars containing boiling 95 per cent. alcohol of sufficient volume to give a final concentration, including the moisture of the sample, of 80 per cent. alcohol. Boiling was continued for 10 minutes, after which the jars were sealed and stored pending analysis. Reductions obtained in similar samples boiled 10, 30, and 60 minutes indicate that complete extraction of the reducing substances was obtained by boiling 10 minutes.

Small amounts of calcium carbonate were added to the first samples to neutralize the acids, but because of the small amounts of acids present and the short period of heating, it seemed that hydrolysis would be negligible. ARCHBOLD (**2**) in a report on work with apples states: "No difference was found in the estimated amounts of sugar in untreated solutions compared with solutions treated with calcium carbonate during the hot extraction or with ammonia during both cold and hot extractions. Hydrolysis during

alcohol extraction is therefore presumed to be negligible." The first season's study showed that the calcium carbonate interfered with the pectin determinations by neutralizing the weak acid used in extracting total pectins. Therefore it was omitted in subsequent samples.

Analysis.—Benzene extractions, alcohol extractions, water extractions, and alkali extractions were made and the cellulose and lignin determinations secured as described by CRIST and BATJER.

Total sugars, sucrose, dextrins, starch, and hemicellulose were determined as outlined by the committee on chemical methods of the American Society of Plant Physiologists (**31**).

Pectins as calcium pectate were determined by the method established by CARRÉ and HAYNES (**6**) and employed by APPLEMAN and CONRAD (**1**).

During the growing seasons of 1930 and 1931, reducing substances were determined by the modified SHAFFER-HARTMANN titration method (**30, 33**). The sugar solutions obtained by taking up the alcohol extracts in water, being quite free of coloring matter, were used directly to avoid loss of sugars which might be thrown down in the clearing process (**4**).

Investigations by PHILLIPS (**28**) indicate that with certain materials the SHAFFER-HARTMANN method gives high values. He cites SULLIVAN (**34**) as finding that iodine liberated in the presence of plant extracts may be absorbed by some constituent of the extract, such as phlorizin, causing an error in the determination of the reducing copper. To test this possibility, reductions were determined on the water solutions of the alcohol extracts of the 1933 samples after being reduced at 80° C. for 30 minutes, first by the modified SHAFFER-HARTMANN titration method in which titration was carried out in the presence of the plant extract; and second, by the volumetric thiosulphate method (**17**) in which the cuprous oxide is separated by means of an asbestos mat in a Gooch crucible and titrated free of the plant extracts. To check further on this point, the filtrate from which the cuprous oxide had been separated was titrated at once by the modified SHAFFER-HARTMANN method, with the results presented in tables I and II.

These data show clearly that with these extracts the modified SHAFFER-HARTMANN method gives values greater than the amounts of copper reduced warrant. Indications are that this difference is due to some substance in the plant extract which does not reduce the copper, but probably behaves as suggested by SULLIVAN (**34**). This substance, whatever its nature, seems to be present in larger amounts in extracts from the earlier samples, which are more highly colored and contain larger proportions of skin to flesh of the fruit.

This test was repeated on cleared solutions taken from apple tissue. Apparently some of the reducing substances were removed by clearing as the amounts of reduced copper were slightly lower. The greatest loss was in

TABLE I

AVERAGE CC. OF 0.1 N SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTION OF FEHLING'S SOLUTION SECURED WITH 50 CC. OF THE ALCOHOL SOLUBLE EXTRACT OF APPLE FRUITS

DATE OF SAMPLING*	UNCLEARED SOLUTIONS							CLEARED SOLUTIONS WITH 0.5 GRAMS OF NEUTRAL LEAD ACETATE PER 100 CC.			
	FIRST TEST			SECOND TEST							
	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	DIFFERENCE BETWEEN THE TWO METHODS	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	SHAFFER-HARTMANN TITRATION OF FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD	COPPER TITRATION PLUS FILTRATE TITRATION	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	SHAFFER-HARTMANN TITRATION OF FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD	COPPER TITRATION PLUS FILTRATE TITRATION
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
May 27				3.4	1.0	2.4	3.4	1.9	0.3	1.5	1.8
June 4	4.7	2.4	2.3	4.8	2.4	2.4	4.8	3.2	2.0	1.0	3.0
10	4.5	3.0	1.5	4.8	3.0	1.7	4.7	3.5	2.6	0.7	3.5
16	6.9	5.7	1.2	6.9	5.3	1.6	6.9	5.7	5.1	0.8	5.9
24	7.9	6.7	1.2	8.0	6.8	1.0	7.0	6.0	6.2	0.4	6.6
30	8.2	7.7	0.5	8.3	7.7	0.7	8.4	7.6	7.4	0.5	7.9
July 7	8.4	8.1	0.3	8.7	8.3	0.6	8.9	8.4	8.0	0.4	8.4
14	9.5	9.5	.	9.9	9.4	0.5	9.9	9.6	9.2	0.4	9.6
25	10.7	9.7	1.0	11.2	10.7	0.6	11.3	11.5	11.3	0.2	11.5
Aug. 9	11.8	11.8	.	11.7	11.4	0.4	11.8	11.0	10.7	0.4	11.1
Sept. 9	14.5	14.3	0.2	14.6	14.0	0.4	14.4	13.9	13.6	0.2	13.8
Oct. 9	13.3	13.5	0.3	13.8	13.4	0.3	13.7	13.4	13.1	0.2	13.3

* 80 grams fresh weight for each sample.

the SHAFFER-HARTMANN titrations and shows that about half of this unknown material was taken from the solutions by clearing. It would seem from these facts that this substance is associated with the skin or pigments in both apple and pear fruits.

Reducing substances in the 1933 samples were determined by the volumetric thiosulphate method, as described (17), except that reduction was carried out at 80° C. for 30 minutes by means of a hot water bath.

TABLE II

AVERAGE CC. OF 0.1 N SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTIONS OF FEHLING'S SOLUTION SECURED WITH 50 CC. OF THE ALCOHOL SOLUBLE EXTRACT OF PEAR FRUITS

DATE OF SAMPLING*		TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD TITRATED BY SHAFFER-HARTMANN METHOD	COPPER TITRATION PLUS FILTRATION TITRATION
		cc.	cc.	cc.	cc.
May	20	1.6	0.3	0.6	0.9
	27	3.2	1.5	1.5	3.0
June	4	3.7	2.2	0.9	3.1
	10	3.1	2.3	0.9	3.2
	16	4.1	2.9	0.6	3.5
	24	3.8	3.0	0.4	3.4
	30	3.7	3.0	0.2	3.2
July	7	5.2	4.6	0.2	4.8
	14	6.3	5.4	0.3	5.7
	25	7.3	6.3	0.3	6.6
August	9	9.6	8.7	0.1	8.8
September	9	15.8	14.7	0.1	14.8
October	9	16.7	16.3		16.3
Novemebr	4	19.0	18.6	0.2	18.8

* 80 grams fresh weight per sample.

Hardness of fruits.—Hardness of the fruits was obtained on each sampling date by means of a pressure tester (20). A plunger of 4/16 inch diameter was substituted for the regulation 5/16 inch one, to permit readings on the early hard fruits. The pressures thus obtained may be converted, approximately, over to 5/16 plunger values, by the factors 1.42 when skin is removed and 1.35 when skin is not removed. These factors were derived by determining the pressure for each plunger on the same fruits. As the ratio varies a little, being greater with the softer fruits, these factors are only approximate, but serve to give an estimate of the hardness of the early fruits.

Procedure and results

During the growing season of 1930, samples for chemical analysis were taken periodically of Kieffer and Bartlett pears and Wagener apples from vigorous, productive trees.

Changes in the amount of lignocellulose, total sugars, reducing sugars, total pectins and soluble pectins, as percentages of the dry weight were

determined. Figure 1 compares graphically the changes in lignocellulose and total sugars in the three fruits studied.

A more detailed story was desired of the changes occurring in the Kieffer fruits. Consequently in 1931 fruits of Kieffer pear were sampled every third day from June 2 until July 17 and less frequently thereafter, as long as any fruits remained on the tree. As many as 1200 fruits were required to furnish enough material for a single sample on the earlier dates and a minimum of 25 fruits was used in each sample.

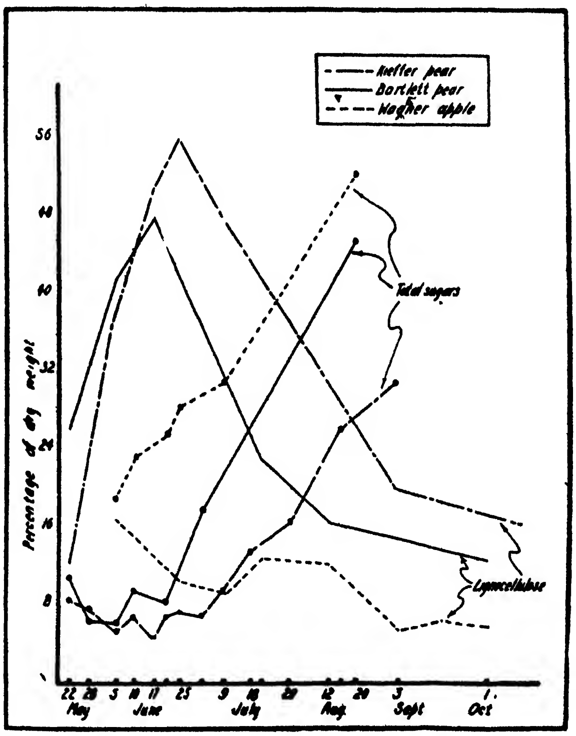


FIG. 1. Seasonal course of total sugars and lignocellulose as percentage of dry weight in fruits of different "grittiness," 1930.

Quantitative determinations of the constituents of the Kieffer pear fruits secured during the growing season of 1931 are presented in table III and graphically in figure 2. The changes in lignocellulose, sugars, and pectins are similar to those secured in 1930.

Changes in relative amounts of lignocellulose in the Kieffer (fig. 1 and 2), consisting of a very rapid accumulation during the first four weeks after fruit setting followed by a less rapid decrease, are identical with those found by CRIST and BATJER (7). The accumulation of lignocellulose is accompanied by a decrease in the amounts of total and reducing sugars, until

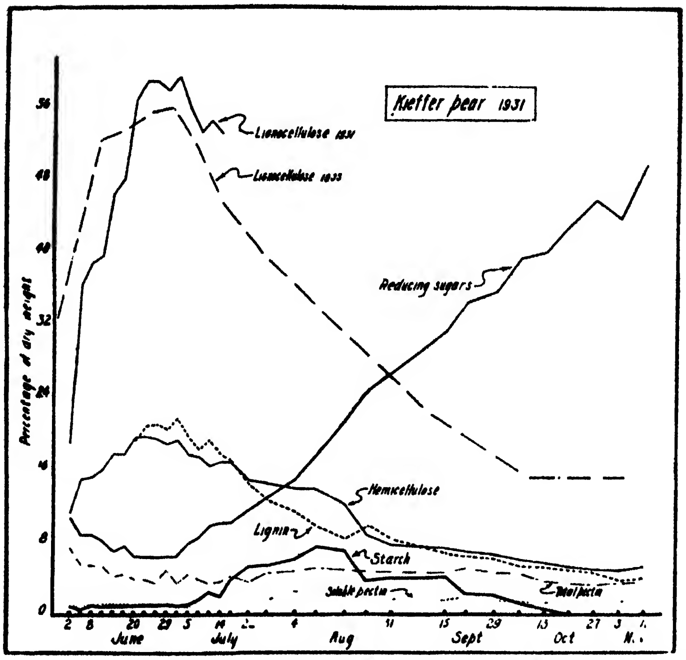


FIG. 2. Seasonal course as percentage of dry weight of several constituents of Kieffer pear fruit, 1931.

a few days before the peak of concentration of lignocellulose is reached. The sugar curves then indicate a piling up of sugars coincident with the decrease in lignocellulose. The difference between total and reducing sugars is reported as sucrose and persists in relatively small amounts. The curve for sugar concentrations could almost be a reciprocal of the curve for lignocellulose both in value and direction. The percentage of total pectins decreases during the early growing season, while soluble pectins increase. After the first part of July, both show a slight gradual increase. The difference between total and soluble pectins is reported as protopectin (6), which goes over to soluble pectins during the life of the pear.

Comparison of changes in the apple and pear fruits

That "grit cells" are composed chiefly of lignocellulose, and that their formation is a result of lignification which may be measured quantitatively by lignocellulose determinations has been established by Crist and Batjer.

As would be expected from their findings, the percentage of lignified tissue does not increase in the "grit cell"-free Wagener apple fruits (fig. 1). Although lignocellulose starts at a concentration equal to that in the pear, it decreases continuously throughout the season, except for a short

TABLE III

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS, 1931, DRY WEIGHT BASIS

DATE OF SAMPLING	MOISTURE	DRY WT. OF FRUIT	DRY WT. OF ALC. EXT.	REDUCING SUGARS	DEXTRINS	STARCH	HEMICELLULOSE	RESIDUE*	LIGNIN	PECTIN	TOTAL PECTIN
	%	%	%	%	%	%	%	%	%	%	%
June 2	84.87	15.13	51.67	10.63	0.84	0.76	11.14	19.07		0.75	7.30
5	84.27	15.73	42.82	8.46	0.61	0.54	14.91	36.20		0.99	5.61
8	83.54	16.46	43.25	8.62	0.54	0.77	15.01	39.10		0.75	5.52
11	83.43	17.57	42.19	8.12	0.64	0.67	15.93	39.79		0.87	5.42
14	81.23	18.77	34.62	6.92	0.62	0.74	17.82	46.22		0.92	4.08
17	78.92	21.08	34.83	7.44	0.80	0.66	17.64	48.15		0.74	4.21
20	78.26	21.74	32.58	6.20	0.42	0.63	19.59	41.74	19.20	1.07	3.95
23	77.63	22.37	29.96	6.25	0.83	0.75	19.63	40.22	20.70	1.10	3.85
26	77.58	22.42	30.50	6.19	0.71	0.66	19.51	41.62	20.80	1.10	3.68
29	77.53	22.47	31.64	6.36	1.00	0.85	18.82	42.87	20.30	0.85	4.75
July 2	76.83	23.17	29.77	6.38	1.07	0.75	19.23	42.90	21.28	0.71	3.68
5	75.85	24.15	33.28	7.94	1.80	0.87	17.75	42.98	19.80	0.71	4.30
8	76.34	23.66	34.79	8.45	.95	1.62	17.50	40.01	18.36	1.19	3.89
11	76.55	23.45	36.67	9.56	1.07	2.02	16.30	38.68	19.24	1.05	3.58
14	77.96	22.04	37.60	10.08	1.44	1.72	16.79	37.96	17.86	1.03	3.64
17	78.60	21.40	37.80	10.00	1.16	3.85	16.98	35.80	17.36	1.00	4.11
22	76.54	23.46	39.53	11.30	1.14	5.19	14.78	32.74	14.45	0.65	3.79
27	77.00	23.00	44.69	12.60	1.60	5.40	14.60	28.97	12.40	1.61	4.34
August 4	78.00	22.00	49.42	14.72	1.75	6.10	13.97	26.66	11.05	2.68	4.62
10	79.57	20.43	54.76	17.79	2.05	7.61	13.77	24.20	9.81	1.27	5.08
18	79.77	20.23	59.86	21.38	1.76	7.06	12.12	21.14	8.36	1.97	5.03
24	80.00	20.00	63.53	24.55	1.26	3.82	8.88	19.14	9.82	1.40	4.61
31	80.36	19.64	64.73	26.73	1.80	4.00	7.80	17.53	8.53	1.80	4.73
September 15	80.91	19.09	71.83	31.40	1.32	4.12	7.65	14.27	6.86	1.61	4.69
21	82.59	17.41	71.90	34.73	1.44	2.11	7.08	14.18	6.54	1.94	5.15
29	82.58	17.42	76.23	35.87	0.99	2.10	6.53	11.97	6.05	1.60	4.58
October 6	82.65	17.35	80.23	39.59	0.93	1.14	5.94	10.79	5.44	1.47	4.41
13	82.18	17.82	80.61	40.17	0.69	0.39	5.79	10.65	5.27	1.42	3.73
20	82.26	17.74	84.72	43.29	0.66	trace	5.38	9.72	4.67	1.26	3.67
27	82.16	17.84	86.49	46.07	0.61	trace	5.03	9.37	4.34	1.76	3.91
November 3	82.62	17.38	87.27	44.76	0.87	0.13	4.85	9.42	3.96	1.62	3.85
10	84.00	17.00	98.85	49.87	0.62	0.25	5.37	10.13	4.22	1.35	3.88

* Fraction of sample remaining after carbohydrate extraction.

period in July. Accumulation of sugars is not delayed as in the pear fruits, but proceeds at a uniform rate from the very start.

Changes in hemicelluloses in the Kieffer pear (fig. 2) follow closely changes in lignin. Although the changes are of a different character in the apple, this relation of hemicellulose to lignin seems to hold, as WIDDOWSON (**37**) also shows a rapid decrease in the percentage of hemicellulose in the early life of Bramley's seedling apple, followed by a less rapid decline during the remainder of the growing season. Changes in starch concentration found in the apple by WIDDOWSON (**37**) and TETLEY (**35**) have the same character as those found in the Kieffer pear.

Changes in the Bartlett pear are similar to those in the Kieffer, but with a general shortening of the whole process. Lignocellulose curves start at a higher concentration, reach a minimum earlier in the season and fall to a lower level than those for the Kieffer (fig. 1). Although total sugars show a decrease for the first two weeks after fruit set, they are less pronounced than in the Kieffer and accumulation of sugars starts about 10 days earlier (fig. 1). The greater amount of total pectins in the Bartlett seems to be due to a greater quantity of protopectin. The character of the pectin changes is much like that found in Kieffer.

Inspection of figures 1 and 2 reveals a critical "point of change" in the pear fruits where those constituents which have been accumulating decrease suddenly, and those materials which have been decreasing, begin to accumulate. This "conversion point" occurs about ten days earlier in the Bartlett fruit than in the Kieffer.

In the Wagener apple there is a rapid accumulation of sugars, showing a steady increase in sucrose, and a gradual decrease in lignocellulose from the very earliest sampling. As in the pear fruits, protopectin goes over to soluble pectin early in the season. The increase in both total and soluble pectins during the last part of the growing season distinguishes pectin changes in the apple qualitatively from those in the pear.

ONSLOW (**27**) and CRIST and BATJER show lignin to account consistently for about one-third of the lignocellulose. These data coincide with their findings and, as would be expected, lignin changes are qualitatively the same as lignocellulose (fig. 2).

Hemicellulose changes, presented graphically in figure 2, are almost identical with lignin changes both in amounts and direction, except for the period of starch concentration from July 17 to August 24. During this time hemicelluloses do not decrease as rapidly as does the lignin and they maintain a difference of about 4 per cent. of the dry weight. It may be significant that this over-rapid decrease in lignin, and slowing of the hemicellulose decline, coincide closely with the high concentration of starch.

TABLE IV

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS IN STORAGE AT 33° F., DRY WEIGHT BASIS

	MOISTURE	DRY WT. OF FRUIT	DRY WT. OF ALC. EXT.	REDUCING SUGARS	DEXTRIN	STARCH	HEMI-CELLULOSES	RESIDUE*	LIGNIN	PECTIN	TOTAL PECTIN
Harvested and placed in storage September 29, 1931											
	%	%	%	%	%	%	%	%	%	%	%
Sept. 29	82.30	17.70		37.92	0.99	0.67	6.39	14.07	6.67	2.32	4.76
Oct. 13	82.42	17.58		38.81	0.86	0.31	6.34	14.99	6.84	2.50	4.64
Nov. 26	82.70	17.30		38.84	0.73	0.45	6.76	15.77	7.29	1.79	5.82
Dec. 17	83.31	16.69		39.98	0.89	0.34	6.04	16.19	7.73	2.07	5.05
Jan. 7	83.13	16.87		40.02	1.30	0.35	6.82	15.52	7.51	1.98	5.88
" 21	84.37	15.63		41.59	1.36	0.31	6.50	16.89	7.72	2.73	6.77
Feb. 11	84.90	15.10		43.97	1.08	0.42	6.42	16.85	7.53	3.19	5.56
" 25	84.30	15.70		42.47	1.24	0.98	5.57	15.75	7.16	3.48	5.32
Harvested and placed in storage October 13, 1931											
Oct. 13	82.44	17.56	79.72	42.14	0.79	0.22	5.55	12.41	5.33	2.22	4.55
Nov. 26	82.54	17.46	83.61	44.26	0.75	0.28	5.77	12.91	4.93	1.65	4.17
Dec. 17	83.00	17.00	85.29	44.70	0.78	0.24	5.84	12.91	6.25	1.81	4.70
Jan. 7	83.10	16.90	85.02	45.20		0.32	5.39	13.64	6.44	1.46	4.73
" 21	84.21	15.79	85.54	45.22	1.16	0.39	6.48	15.52	7.16	2.00	
Feb. 11	83.60	16.40	83.92	45.85	1.16	0.87	5.60	14.31	7.12	2.53	5.70
Feb. 25	83.57	16.43	84.50	45.74	0.90	0.35	5.49	13.64	6.09	2.73	4.54
Harvested and placed in storage October 27, 1931											
Oct. 27	82.40	17.60		45.79	0.68	0.18	4.90	11.87	5.13	1.30	3.88
Nov. 26	82.74	17.26		46.16	0.63	0.28	5.04	11.30	5.36	1.01	4.00
Dec. 17	83.44	16.56		48.26	0.62	0.28	5.89	12.43	5.51	1.87	4.00
Jan. 7	83.72	16.28		48.69	1.11	0.46	5.40	12.20	5.77	1.62	5.52
" 21	84.16	15.84		50.75	0.90	0.22	4.74	12.11	5.19	2.27	5.15
Feb. 11	84.22	15.78		49.10	0.80	0.68	4.08	12.53	5.47	2.10	5.06
" 25	83.78	16.22		47.59	0.81	0.63	4.53	12.75	5.48	2.51	4.26

* Fraction of sample remaining after carbohydrate extraction.

Starch remains insignificant (less than 1 per cent.) until early July when it begins to accumulate rapidly, reaching a concentration of 5 per cent. This high concentration is maintained until the middle of August, after which a uniform decline occurs and starch again becomes insignificant about the middle of October.

Dextrins and soluble starches do not become important at any time. They do, however, follow the general trend of the starches, with concentrations varying from 0.5 to 1.5 per cent. of the dry weight.

Carbohydrate residue curves are similar to lignocellulose curves. Analyses show this residue to be composed almost entirely of lignocellulose.

RIVIÈRE and BAILHACHE (**32**) report that ripening, as measured by the sugar content, is progressive from the stem end to the calyx end in the three varieties of pears studied; namely, Beurré Hardy, Angouleme, and Comice. If this is true in the Kieffer pear, sampling which includes the whole fruit would be more representative of the sugar content than that taking only the mid-section. For this reason, and to permit the expression of the various constituents in absolute quantities per fruit, the samples taken in 1933 of Kieffer pear friuts and Wagener apple friuts from the college orchard comprised whole fruits from which the loculi of the carpels with their contents were removed. The average weights and volumes of the fruits were determined at each sampling.

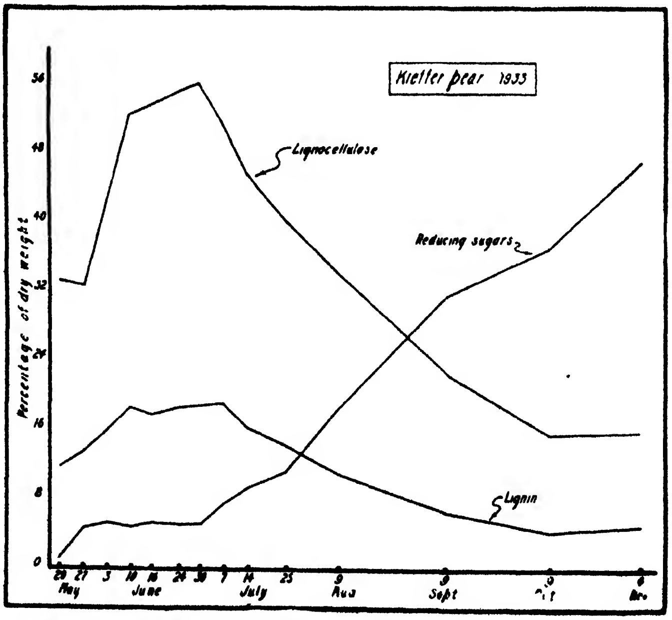


FIG. 3. Seasonal course of reducing sugars, lignocellulose, and lignin as percentage of dry weight. Kieffer pear fruit, 1933.

The findings secured in 1933 are presented graphically in figures 3 and 4 on a dry weight basis and in figures 5 and 6 as absolute amounts of

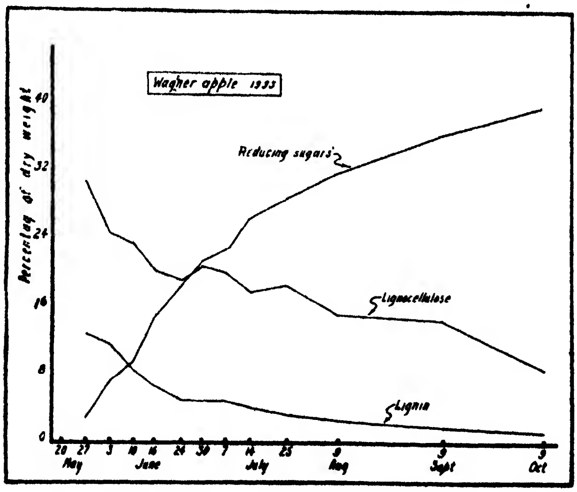


FIG. 4. Seasonal course of reducing sugars, lignocellulose, and lignin as percentage of dry weight. Wagener apple fruit, 1933.

the constituents per fruit. On a dry weight basis the findings are in accord with those of 1930 and 1931. On an absolute amount per fruit basis, an entirely different picture of the changes in the constituents is obtained.

Inspection of figures 3 and 5 reveals the deceptiveness of expressions on the basis of percentage of dry weight. None of the constituents of the

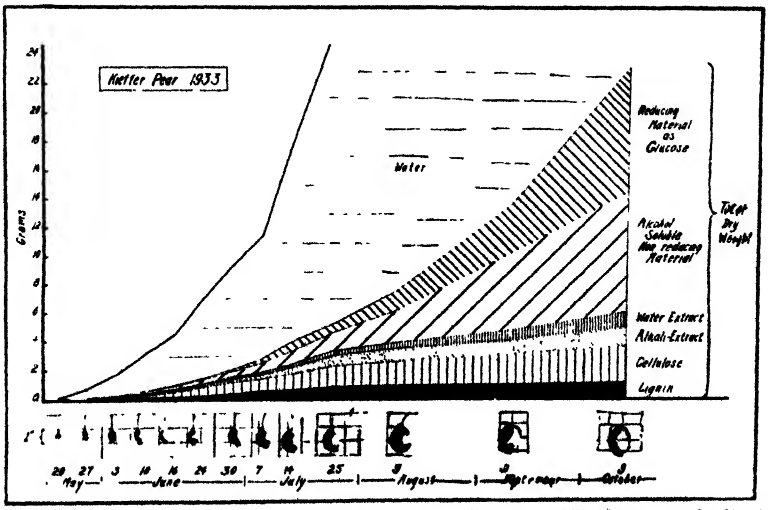


FIG. 5. Seasonal course of actual weight of constituents. Kieffer pear fruit, 1933.

TABLE V

PERCENTAGE COMPOSITION OF KIEFFER PEAR AND WAGENER APPLE, 1933, DRY WEIGHT BASIS

DATE OF SAMPLING		KIEFFER PEAR				WAGENER APPLE			
		DRY WEIGHT PERCENTAGE OF FRESH WEIGHT	PERCENTAGE OF DRY WEIGHT			DRY WEIGHT PERCENTAGE OF FRESH WEIGHT	PERCENTAGE OF DRY WEIGHT		
			LIGNO-CELLULOSE	LIGNIN	REDUCING MATERIAL AS DEXTROSE		LIGNO-CELLULOSE	LIGNIN	REDUCING MATERIAL AS DEXTROSE
		%	%	%	%	%	%	%	%
May	20	11.1	33.8	11.7	1.0				
"	27	12.2	32.6	13.4	4.5	13.5	30.5	12.5	2.8
June	4	15.9	42.3	15.8	5.2	12.7	24.3	11.8	7.2
"	10	19.2	52.2	18.7	4.7	12.7	23.3	8.2	9.1
"	16	21.3	53.5	17.8	5.3	13.9	20.2	6.5	14.8
"	24	22.0	55.6	18.4	5.3	14.4	19.2	4.9	18.4
"	30	22.0	56.0	18.9	5.3	13.9	20.6	4.7	21.7
July	7	23.4	51.5	17.4	7.9	14.2	20.0	4.8	23.1
"	14	22.0	45.5	16.2	9.6	14.0	17.9	4.0	26.3
"	25	20.0	40.2	14.2	11.2	14.5	18.8	3.3	29.0
August	9	18.7	34.3	11.0	18.2	14.2	15.2	2.4	31.7
September	9	18.5	22.3	6.9	31.3	15.1	14.5	1.9	36.6
October	9	17.4	15.5	4.3	37.2	13.5	8.9	1.1	39.1

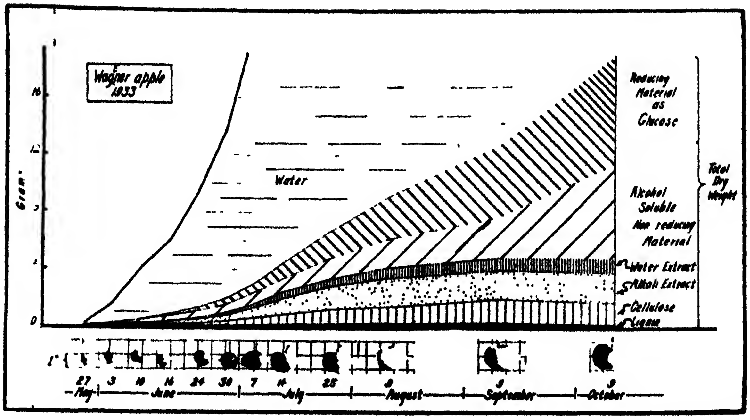


FIG. 6. Seasonal course of actual weight of constituents. Wagener apple fruit, 1933.

pear fruits decreases, but each one actually increases throughout the growing season. Figure 5 shows clearly that the apparent increase and decrease in lignocellulose, when expressed on a dry weight basis, is due only slightly to changes in the rate of accumulation of lignin and cellulose, and principally to changes of the total dry weight. The chief variable of the dry

TABLE VI

CONSTITUENTS OF KIEFFER PEAR IN GRAMS PER FRUIT, 1933

Date of sampling		Moisture	Dry weight	Alcohol soluble non-reducing extract	Reducing material as dextrose	Water extract	Alkali extract	Lignocellulose	Lignin
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
May	20	0.08	0.01	0.003	0.0001	0.0019	0.0012	0.0011	0.0013
"	27	0.71	0.10	0.040	0.0040	0.0103	0.0121	0.0189	0.0132
June	4	1.52	0.28	0.095	0.0149	0.0166	0.0425	0.0306	0.0905
"	10	2.64	0.62	0.162	0.0293	0.0321	0.0839	0.2101	0.1169
"	16	3.53	0.95	0.232	0.0508	0.0454	0.1202	0.3405	0.1700
"	24	5.13	1.44	0.325	0.0761	0.0645	0.1839	0.5387	0.2662
"	30	7.37	2.07	0.451	0.1093	0.1015	0.2457	0.7721	0.3925
July	7	8.85	2.70	0.608	0.2131	0.1103	0.3438	0.7769	0.6160
"	14	13.83	3.90	0.927	0.3727	0.1724	0.4375	1.1419	0.6336
"	25	20.92	5.23	1.369	0.6465	0.2799	0.6995	1.499	0.8202
August	9	33.34	7.66	2.320	1.3958	0.3596	0.9312	1.7843	0.8474
September	9	60.45	13.71	5.433	4.3308	0.6299	0.9611	2.1299	0.9608
October	9	109.86	23.14	9.773	8.6350	1.0775	1.3871	2.6135	0.9943

weight is the alcohol soluble substances, especially reducing sugars. Lignin and cellulose accumulate faster during May and June than the other dry weight constituents, making a great relative increase of these substances

for this period. They continue to accumulate, but at a little slower rate, during the rest of the growing season. However, the rapid accumulation of alcohol soluble substances beginning about July 1 increases the total dry weight of the fruits so quickly that a relative expression of lignocellulose (fig. 3) indicates, unless cautiously considered, a sudden and rapid decrease of this material.

TABLE VII

CONSTITUENTS OF WAGENER APPLE IN GRAMS PER FRUIT, 1933

DATE OF SAMPLING	MOISTURE	DRY WEIGHT	ALCOHOL SOLUBLE NON-REDUCING EXTRACT	REDUCING MATERIAL AS DEXTROSE	WATER EXTRACT	ALKALI EXTRACT	LIGNO-CELLULOSE	LIGNIN
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May 27	0.26	0.04	0.0160	0.0011	0.0049	0.0065	0.0128	0.0052
June 4	1.64	0.20	0.0881	0.0145	0.0139	0.0346	0.0489	0.0237
" 10	4.13	0.53	0.2247	0.0483	0.0427	0.1063	0.1245	0.0438
" 16	5.98	0.84	0.2985	0.1263	0.0645	0.2018	0.1728	0.0557
" 24	9.19	1.33	0.3740	0.2465	0.1257	0.3093	0.2560	0.0656
" 30	13.61	1.89	0.4775	0.4135	0.2003	0.4573	0.3917	0.0903
July 7	19.53	2.97	0.6710	0.6871	0.3213	0.7377	0.5973	0.1431
" 14	30.40	4.43	0.9898	1.1687	0.4848	1.1137	0.7940	0.1789
" 25	42.36	6.69	1.5445	1.9462	0.5712	1.6000	1.2585	0.2184
August 9	58.50	9.10	2.1508	2.9048	0.7550	2.0561	1.3859	0.2233
September 9	81.33	13.77	4.7782	5.0919	0.9004	2.0052	2.0147	0.2696
October 9	126.24	18.96	8.6483	7.6017	1.0667	2.2098	1.7275	0.2176

During May and June the increase in size of the fruit is due largely to the formation of new cells and at this time cell wall material accounts for most of the dry weight of the fruit. After the last of June, increase in fruit size is due to expansion of the already formed cells and to enlargement of the intercellular spaces (TETLEY, **36**). This behavior would occasion a progressive decrease in the proportion of cell wall to cell contents, thereby showing a less rapid increase in cell wall materials (lignocellulose) during the remainder of the growing season. Alcohol soluble materials are present chiefly in the vacuoles of the cells and, as would be expected, the larger the cells the greater the proportion of cell inclusions to cell wall constituents. Thereafter, it would seem that the great increase in dry weight is due to cell inclusions and should be considered separately from the cell wall constituents.

Hardness.—Data on hardness of fruits in terms of pounds pressure as determined by the standard pressure tester are presented in figure 8 and table IX.

TABLE VIII

WEIGHT AND VOLUME OF KIEFFER PEAR AND WAGENER APPLE FRUITS, 1933

DATE OF SAMPLING		KIEFFER PEAR			WAGENER APPLE		
		AV. WEIGHT	AV. VOLUME*	SP. GR.	AV. WEIGHT	AV. VOLUME*	SP. GR.
		gm.	*cc.*		*gm.*	*cc.*	
May	20	0.09	0.09	1.00	......	......	....
"	27	0.75	0.73	1.02	0.30	0.30	1.00
June	3	1.92	1.84	1.04	1.84	1.89	.97
"	10	3.51	3.22	1.09	4.66	4.91	.94
"	16	4.82	4.45	1.08	6.82	7.63	.89
"	24	7.08	6.62	1.07	10.52	11.53	.91
"	30	10.16	9.27	1.09	15.50	17.72	.87
July	7	12.34	11.53	1.07	22.50	25.76	.87
"	14	18.70	17.76	1.05	34.83	40.00	.87
"	25	27.60	26.15	1.05	49.05	55.70	.88
August	9	42.80	40.60	1.05	67.80	77.41	.87
September	9	77.50	75.00	1.03	95.10	111.42	.85
October	9	138.20	125.00	1.10	145.20	164.00	.88

* Volume determined by displacement.

TABLE IX

HARDNESS OF KIEFFER PEAR AND WAGENER APPLE FRUITS, 1933

DATE OF SAMPLING		KIEFFER PEAR				WAGENER APPLE			
		MEAN	S.D.*	P.E.†	CORRECTED FOR GOV'T. PRESSURE TESTER‡	MEAN	S.D.*	P.E.†	CORRECTED FOR GOV'T. PRESSURE TESTER‡
		lb.			*lb.*	*lb.*			*lb.*
June	10	20.52	1.58	1.06	27.6	20.04	1.75	1.18	27.5
"	16	22.90	1.77	1.19	30.9	19.52	1.06	0.71	26.3
"	24	24.11	0.38	0.25	32.5	20.65	1.78	1.20	27.8
"	30	23.44	1.67	1.12	31.5	22.31	2.14	1.44	30.1
July	7	25.44	1.60	1.07	34.2	20.71	1.42	0.95	28.0
"	14	24.27	1.21	0.82	32.6	20.08	0.87	0.58	27.0
"	25	22.40	0.35	0.23	30.2	18.38	2.43	1.63	24.8
August	9	20.79	1.30	0.87	28.0	16.63	0.80	0.53	22.4
September	9	17.29	1.10	0.74	23.3	12.00	1.55	1.04	16.2
October	9	12.84	0.40	0.26	17.2	10.80	0.79	0.53	14.5

* Standard deviation = $\sqrt{\frac{\text{Summation } X^2}{n} - M^2}$.

† Probable error = S.D. × .6745.

‡ Times factor 1.35 to compare with 5/16 regulation size plunger.

Storage studies

For storage studies, Kieffer fruits were picked at three dates: the first lot on September 29, before the normal picking date for this variety; the second on October 13, about the regular picking time; and the last lot on October 27, later than they are usually harvested. The fruits were picked into baskets and placed immediately in cold storage at 33° F. Samples for chemical analyses were taken monthly during storage. Notes on the condition of the fruits were made at time of sampling.

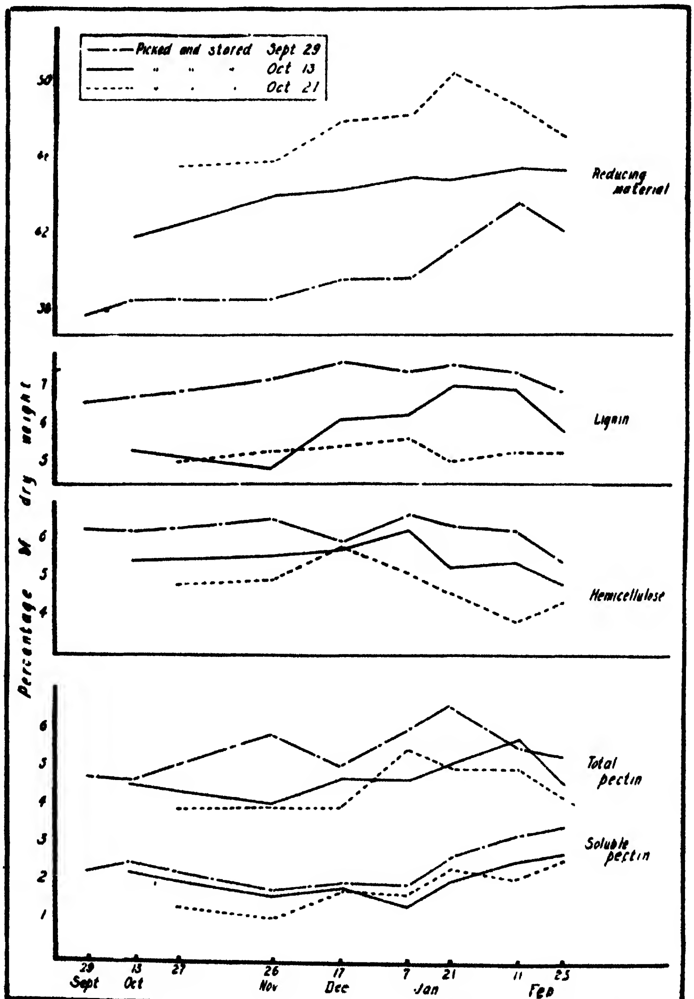


FIG. 7. Percentage change in constituents of Kieffer pear fruits in storage at 33° F., 1931–32.

EMMETT (**13**) in an investigation of changes in pear fruits found that "loss of weight in storage is due chiefly to transpiration." In this study in 1931–1932 the pear fruits showed a loss of 1.5 per cent. of their fresh weight per month. The dry weight concentration of the fruit actually decreased with a corresponding increase in precentage of moisture. In spite of this increase in percentage of moisture the major loss in weight of the fruit is moisture. Thus, paradoxically, the moisture decreases in amount while it is increasing in percentage.

Of the three picking dates represented, October 13 proved to be the best for storage. Determinations of reducing materials, soluble pectin, total pectins, hemicelluloses, and lignin made on these fruits during storage are presented in table VII and figure 7. On the dry weight basis these data show increase in all constituents except hemicellulose during storage. WIDDOWSON found hemicelluloses to decrease in apples in storage. The later-picked fruits had a higher concentration of sugars and lower concentration of lignin, pectins, and hemicelluloses. The intermediate picking was intermediate in all these respects. It may be significant that sugars show a sharp rise followed by a sharp decrease in the early and late pickings. The last picking indicates this break first and this is the inverse order of their keeping quality in storage. This also seems to be the case with total pectins which disappear as pears become overripe and start to break down. EMMETT found this to be the case in Bartlett pears. In general, chemical changes during the ripening and breakdown in storage

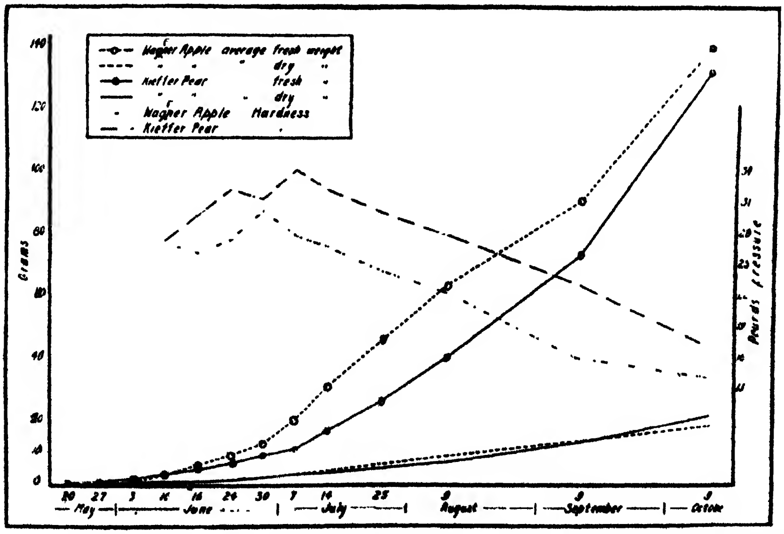


FIG. 8. Dry weight, fresh weight, and hardness of Kieffer pear and Wagener apple fruit, 1933.

are similar in pears and apples. The hemicelluloses seem to be the original source of respirable material.

The Kieffer pear fruits picked on October 13 with a pressure of 14.8 pounds skinned or 18.2 pounds unskinned, kept much better in storage than the later or earlier picked fruits. The reducing material content at that time was 42 per cent. of the fruits' dry weight, although as MAGNESS (**19**) states, "differences in chemical composition due to variations in growing conditions are so great in relation to those due to stage of maturity that any picking test based on chemical composition would prove unsatisfactory."

Discussion and conclusions

Assuming that the course of events in the process of lignification occurs as diagrammed, the constituents in order of their complexity would follow the scheme outlined by ONSLOW (**26**). First, some of the sugar becomes oxidized to polyuronic acids, such as galacturonic and glucuronic, which may combine with condensation products of the sugars, such as arabinose and galactose, to form pectic substances. These pectic substances, then, by decarboxylation form five-carbon sugars, such as arabinose, xylose, and some hexoses and uronic acids which together make up the hemicelluloses.

The hemicelluloses may go to lignin, a substance having an uncertain empirical formula. NORRIS and SCHRYVER (**24**) were able to produce some hemicellulose-like material by treating a pectin preparation. CANDLIN and SCHRYVER (**5**), also by treating pectin with alkalis, secured hemicellulose, similar to that isolated from wood in all respects, and some unidentified residues which they state might possibly form combinations with cellulose to produce lignocellulose.

The cellulose seems to be composed of pure glucose and probably is formed directly by condensation of glucose (**27**, p. 67). Lignocellulose has a composition of about 60 per cent. cellulose and 40 per cent. lignin (**7, 27**). Two general theories as to the formation of lignin exist (**16**, p. 49); first, that cellulose of the cell wall is converted directly to lignin or lignocellulose; and second, that materials other than cellulose are lignin precursors.

ONSLOW (**26**, p. 69) supports the first view: "as the cells in plants grow older the walls usually become lignified; that is, part of the cellulose becomes converted to lignocellulose." KÖNIG and RUMP (**18**) also suggest the conversion of cellulose to lignin.

The changes in the relative amounts of the constituents, as indicated in figures 1, 2, 3, and 4, strengthen the hypothesis presented in the lignification diagram. We may consider that the sugars, which the leaves are supplying to the fruits, are being converted to pectins and then to hemicelluloses and finally to lignocellulose. The data show a relative decrease in

sugars during lignification in Kieffer fruit. In the Bartlett fruit with less lignification, the accumulation of sugars is retarded less; and in the apple, with no lignification, no checking of the concentration of sugars occurs. We should expect the pectins, being intermediate products, to be more uniform; and hemicelluloses, because of their greater complexity, to vary more with the end product. This is borne out by their relative concentrations (fig. 2). Associated with the decrease in lignocellulose is an intermediate decrease in hemicelluloses and a sharp increase in sugars. From the graphs showing changes as percentage of the dry weight it is easy to imagine the lignocellulose being broken down to hemicelluloses and then to sugars.

Magness (19), referring to his work with Bartlett pears, concludes that "as fruits ripen on the tree, much material other than starch is converted into sugars." Murneek (22) suggests that in the apple, hemicelluloses are a source of sugar for the maturing fruit. Crist and Batjer (7) suggest a destruction of lignocellulose, and from histological studies, find the clusters of "grit cells" apparently becoming smaller as there is more unlignified tissue between the clusters. These findings seem to support the possibility of a breaking down of the more complex materials to simpler ones in Kieffer pear fruits during the latter part of the growing season.

If the same data are plotted (fig. 5) as absolute amounts per fruit during the growing season, it becomes difficult to imagine any of the constituents breaking down. The data show clearly an increase in every fraction. Hemicellulose changes are almost identical with lignin changes (table III, fig. 2) and would, if presented as absolute amounts per fruit, show the same increase during the growing season. With these particular data, the possibility of hemicellulose supplying sugar to the maturing Kieffer pear fruit is not supported. The case may be quite different in the apple, however, as an inspection of figure 6 reveals a slight decrease during the latter part of the growing season in total amounts of lignin and cellulose. As the absolute amount of lignocellulose in the pear fruit does not decrease, but actually increases, the destruction of lignocellulose (7) could be accounted for by its being formed in new parts of the fruit, as nearer the periphery, faster than it is destroyed in the more concentrated areas. The writer doubts that actual destruction of lignocellulose occurs. An apparent decrease in size of the "grit cell" clusters may be due to the clusters being pushed farther apart as the fruit increases in size, due to increase in size of individual cells in the latter part of the growing season, thus distributing the stone cells over a greater area.

It therefore becomes apparent that there is no basis for the support of the supposition that lignocellulose is being converted over to sugars or

to any other material. However, it does seem quite probable that the building up of lignocellulose is through these intermediate materials.

It is evident, therefore, that the amount of grit cells of pears depends principally on the extent of grit cell formation during early stages of the formation of the fruit and apparently is not reduced by changes taking place in the grit aggregation during the latter part of the growing season or during ripening. This in turn means that the process is not likely to be materially influenced by cultural or handling practices and bears out the suggestion of Crist and Batjer that only through his choice of varieties does the pear grower have any considerable control over this more or less objectionable characteristic of pear fruits. A distinction between absolute amount and apparent grittiness is evident when the fruit becomes soft, as when allowed to ripen on the tree, permitting the grits to separate easily from the pulp and making them more noticeable. A fruit ripening on the tree is increasing more rapidly in substances other than grit cells, although it is actually increasing slightly in absolute amounts of grit cells. In apparent grittiness it is increasing greatly. This accounts for the popular impression that "grittiness" increases when pear fruits are allowed to ripen on the tree.

Summary

1. Existing data (and the first two years' results of this study) showed such a tremendous decrease of the percentage of lignocellulose (stone cells) accompanied by an equally great increase of reducing materials, in maturing Kieffer pear fruits, that it suggested that part of the lignocellulose had been converted to reducing substances. Investigations of these changes in the Bartlett pear, a fruit in which lignocellulose occurs in smaller quantities, showed that as a percentage of the dry weight the lignocellulose began to decrease, with a corresponding increase in reducing substances, about 10 days earlier than in the Kieffer. Similar studies of the Wagener apple, a fruit which contains very little lignocellulose, indicated decreases of lignocellulose and accumulation of reducing substances occurring in the first samples taken soon after petal fall. These results strengthened the supposition that lignocellulose may be converted to reducing materials.

2. During the growing season of 1933, changes in absolute amounts of these materials in the Kieffer pear and Wagener apple were determined. These determinations show that there was no actual decrease in lignocellulose, but because of the great increase of alcohol soluble materials, the *percentage* of lignocellulose decreased rapidly. The findings in 1933 indicate that lignified tissue does not break down to form less complex materials in these fruits during growth.

3. Calculated changes in composition may be misleading when presented as percentages. Total "grit" in pear is not reduced during ripening, but "grittiness" is masked by the increased amounts of other constituents of the fruit.

4. There is evidence, however, that the sugars are built up, through the compounds studied, to lignified tissue.

5. In storage, hemicelluloses decreased more than any other constituents; this suggests that these materials may be the source of respirable substances for the fruit after its removal from the tree.

6. The modified Shaffer-Hartmann titration method, employed for determining amounts of reducing material during the first two years' study, was found to give higher values on young pear and young apple fruit extracts than the amounts of copper reduced warrants. The high proportion of skin to flesh in the samples taken when the fruits were small is so closely associated with these unwarranted high values that adsorption of the iodine reagent by some material in the skin is indicated.

7. The suggestion of Crist and Batjer that the grower has little control, other than variety selection, over the "grittiness" of his pear fruits, is supported.

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STUDIES OF THE EFFECT OF ARTIFICIAL WIND ON GROWTH AND TRANSPIRATION IN *HELIANTHUS ANNUUS*

E. V. MARTIN AND F. E. CLEMENTS

(WITH TEN FIGURES)

Introduction

Although considerable attention has been given to the influence of wind on transpiration, the results that have been obtained are very conflicting. Practically all control experiments with this factor have been in the region of very low velocities. KNIGHT (**12**) found that a wind velocity of 7 meters per minute (0.26 miles per hour) caused an increase in the rate of transpiration of about 50 per cent. STÅLFELT (**21**), using single detached leaves suspended in an analytical balance case, obtained a very rapid rise with wind up to a velocity of 10 meters per minute, a slower increase from there to 30 m./min., but no further increase as the wind rose to 60 m./min. (2.24 miles per hour). This maximum increase was 140 per cent. of the value with no wind. FIRBAS (**8**), who employed cut shoots under a wind velocity of 3.75 m./sec., obtained similar results with low rates of transpiration, but with high rates (shoots in direct sunlight) wind actually caused a decrease. SEYBOLD (**18, 19, 20**) concluded that wind has no influence on stomatal transpiration but only on the cuticular component, the result being that only those plants with a high cuticular transpiration, namely hydrophytes, showed an appreciable increase in the transpiration rate due to the action of wind.

Observations on water losses of plants under natural conditions have also yielded conflicting results. COPELAND (**6**) found an increase in the transpiration rate of cocoanut palm in full sunlight of about 100 per cent. with a wind velocity estimated at 5 miles per hour. Others (**4, 9**) have noted an increase with velocities up to 8 miles per hour, but a retardation with higher velocities. WILSON (**22**) observed that the rate of transpiration of some Australian plants was checked when the wind velocity rose to 20 miles per hour. BRIGGS and SHANTZ (**2, 3**), however, concluded from their studies of the correlation between environmental factors and transpiration rates for the growing season that only from 2 to 6 per cent. of the water loss could be attributed directly to the action of wind.

Nearly all the control work in this field has been with cut shoots or leaves, which rarely give dependable results, while that with rooted plants has for the most part been under natural conditions with no attempt to control the wind velocity. A combination of these methods has been utilized

in the present work by growing plants in cans but under otherwise natural conditions and producing artificial wind by means of large fans.

These fans have also been employed in a study of the effect of continuous wind upon plant growth. Although the depressing effect of wind has long been recognized, very few measurements of a quantitative nature have been made. BLANCHARD (1) compared the growth and yield of two orchards of Eureka lemons and found that the factor of improvement in yield due to protection from wind was 5 to 7 and in size of trees about 3. FINNELL (7) has grown marigolds under a constant wind velocity of 15 miles per hour and found that it caused a reduction of about 50 per cent. in dry weight of material produced.

I. Effect of wind on transpiration; short time series

METHODS

For all series, plants of *Helianthus annuus* were grown from seed in water tight, cylindric, galvanized iron cans 8 in. in diameter and 10 in. in height. Each of these was fitted with a removable lid that had in its center a circular opening 2 in. in diameter. The soil used was a good loam of as nearly uniform texture and moisture as was feasible. The holard was set at approximately 65 per cent. of saturation (or 28 per cent. of the dry weight of the soil), and the total amount of water in each can was kept within a few per cent. of this value throughout the growing period as well as during the test series. When necessary, water was added through a glass tube extending into a layer of gravel about 1 in. thick in the bottom of the can. When the plants were 6 to 8 weeks old, the containers were sealed by adding a layer of sand about ¾ in. thick on top of the soil, filling the opening in the lid with non-absorbent cotton, and corking the glass tube. The efficiency of this seal was tested by means of controls without plants. Weighings made on a torsion balance to the nearest gram during the time the experiments were in progress showed that these containers seldom lost a detectable amount.

Wind was produced by fans taken from Pierce-Arrow and Packard motor cars, mounted on specially constructed supports, and driven by ⅓ h.p. induction motors. The wind velocity was regulated partly by pulley ratio and partly by adjusting the distance of the fans from the plants. Velocities from about 0.5 to 25 miles per hour were obtainable with this installation and measurements were made by means of a small fan type anemometer.

In all series, two sets of four plants each were used, one serving as a test set, the other as a control. The two sets were selected from a large group in order that they might be as nearly alike as possible. To obtain conditions sufficiently calm for these experiments, it was necessary to work inside the greenhouse, since there is practically always some movement of

TABLE I

TRANSPIRATION RATES IN GRAMS PER SQUARE DECIMETER PER HOUR FROM SERIES TAKEN INSIDE GREENHOUSE

DATE		NO WIND		WIND = 0.8 M.P.H.					NO WIND		WIND
5/8/35	Time interval	9:30–10:30	10:30–11:30	11:30–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:00	5:00–8:00	8:00 P.M. 8:30 A.M.
	T_a*	0.512	0.539	0.678	0.892	1.08	1.10	1.33	0.787	0.308	0.152
	T_b	0.499	0.544	0.859	1.23	1.38	1.47	1.81	0.727	0.272	0.189
	T_b/T_a	0.98	1.01	1.25	1.38	1.98	1.34	1.36	0.92	0.88	1.24

DATE		NO WIND		WIND = 2.1 M.P.H.						NO WIND	WIND
5/9/35	Time interval	8:30–9:30	9:30–11:30	11:30–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:00	5:00–9:30	6:30 P.M. 8:30 A.M.
	T_a*	0.535	0.605	0.757	0.854	0.850	0.542	0.516	0.445	0.160	0.108
	T_b	0.578	0.593	1.05	1.25	1.16	0.637	0.711	0.548	0.146	0.136
	T_b/T_a	1.08	0.98	1.39	1.46	1.37	1.18	1.38	1.23	0.91	1.26

DATE		NO WIND		WIND = 5.5 M.P.H.					NO WIND		WIND
5/10/35	Time interval	8:30–10:15	10:15–11:30	11:30–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:15	5:15–8:30	8:30 P.M. 8:00 A.M.
	T_a*	0.344	0.707	0.972	1.20	0.682	0.717	1.09	0.595	0.213	0.127
	T_b	0.340	0.710	1.57	1.59	0.856	1.01	1.50	0.540	0.195	0.159
	T_b/T_a	0.99	1.00	1.61	1.32	1.27	1.41	1.38	0.91	0.91	1.25

DATE		NO WIND		WIND = 9.5 M.P.H.					NO WIND		WIND
5/11/35	Time interval	8:00–9:40	9:40–11:40	11:40–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:30	5:30–8:30	8:30 P.M. 7:30 A.M.
	T_a*	0.590	1.52	2.24	2.29	2.63	2.34	1.64	0.676	0.161	0.122
	T_b	0.572	1.57	4.44	3.21	3.98	3.16	2.25	0.568	0.136	0.179
	T_b/T_a	0.97	1.03	1.98	1.40	1.51	1.35	1.37	0.84	0.84	1.47

* T_a and T_b are the transpiration rates of the two sets of plants *a* and *b*, while their ratio is given by T_b/T_a. Set *a* is the control and has no wind at any time, while *b* is the test set and has wind during the periods indicated.

TABLE I—(*Continued*)

TRANSPIRATION RATES IN GRAMS PER SQUARE DECIMETER

DATE		NO WIND			WIND = 16 M.P.H.				
	Time interval	8:30–9:30	9:30–10:30	10:30–11:50	11:50–12:05	12:05–12:30	12:30–2:00	2:00–2:30	2:30–3:30
5/14/35	T_a*	1.05	0.95	1.16	1.18	0.99	1.11	0.86	0.75
	T_b	1.01	1.07	1.07	2.81	1.23	1.53	1.29	1.18
	T_b/T_a	0.96	1.13	0.92	2.38	1.24	1.38	1.50	1.57

		NO WIND		WIND = 2.6 M.P.H.			NO WIND	
	Time interval	9:30–10:10	10:10–10:48	10:48–11:03	11:03–11:38	11:38–12:41	12:41–1:34	1:34–2:38
5/15/35	T_a	0.99	0.95	0.79	0.80	0.69	0.79	1.22
	T_b	1.05	0.89	1.41	0.96	0.91	0.71	1.19
	T_b/T_a	1.06	0.94	1.78	1.20	1.32	0.90	0.97

		NO WIND	WIND = 0.7 M.P.H.			NO WIND			
	Time interval	8:00–9:44	9:44–9:59	9:59–10:37	10:37–11:34	11:34–11:49	11:49–1:13	1:13–4:59	4:59–5:45
5/16/35	T_a	1.15	1.97	2.08	2.49	2.89	2.85	2.13	1.10
	T_b	1.20	2.44	2.44	3.16	2.66	2.66	2.06	1.28
	T_b/T_a	1.04	1.24	1.17	1.27	0.92	0.93	0.97	1.16

* T_a and T_b are the transpiration rates of the two sets of plants *a* and *b*, while their ratio is wind during the periods indicated.

air in the open. The greenhouse measures 39 ft. × 16 ft., and hence is quite large enough for the purpose. Stomatal openings were determined at regular and frequent intervals during each series by means of the well known alcohol method (LLOYD, **14**). Leaf areas in square decimeters were determined by multiplying the product of the length and width of each leaf in centimeters by the factor 0.0134 (CLEMENTS and GOLDSMITH, **5**).

RESULTS

The transpiration measurements from seven representative series are given in table I, in which T_a denotes the rate of transpiration of the control set of plants in grams per square decimeter per hour, T_b that of the test set, and T_b/T_a the ratio of the rate of water loss of *b* to that of *a*. These results are shown graphically in figures 1 and 2, which portray the transpiration rate of the test set in percentage of that of the control. Each series began in the morning with one or more periods during which neither set had any wind, followed by several hours of wind, a second check period of two, and

TABLE I—(*Continued*)

PER HOUR FROM SERIES TAKEN INSIDE GREENHOUSE

No wind		Wind	No wind
3:30–6:00	6:00–8:45	8:45 P.M.–8:50 A.M.	8:50 A.M.–10:15 A.M.
0.524	0.133	0.151	0.95
0.510	0.177	0.302	0.94
0.97	1.33	2.00	0.99

Wind = 5.4 M.P.H.				No wind	
2:38–2:53	2:53–3:23	3:23–3:53	3:53–5:18	5:18–5:33	5:33 P.M.–8:00 A.M.
1.13	1.23	0.92	0.91	0.70	0.170
2.14	1.55	1.05	1.32	0.59	0.166
1.89	1.26	1.14	1.45	0.84	0.98

Wind = 16 M.P.H.					No wind		
5:45–6:00	6:00–6:15	6:15–6:30	6:30–8:15	8:15 P.M.–8:28 A.M.	8:28–8:44	8:44–9:03	9:93–10:06
1.04	0.70	0.475	0.219	0.250	1.44	1.54	1.96
2.08	0.92	0.703	0.386	0.384	1.10	1.44	1.75
2.00	1.31	1.48	1.76	1.54	0.76	0.93	0.89

given by T_b/T_a. Set *a* is the control and has no wind at any time, while *b* is the test set and has

finally usually wind again during the night. For all series, the losses of the two sets during the initial check periods were the same within a few per cent., but when the wind was turned on the test set, its transpiration rate invariably rose with respect to that of the control, the increase depending on the wind velocity.

For the low velocity winds, up to about 2 miles per hour, the transpiration rate increased from 20 to 30 per cent., and this value was maintained approximately constant during the entire time the wind was turned on. For higher velocities, however, there was a comparatively high initial rise lasting no more than 15 minutes and in many cases probably less, followed by a much lower value, after which there was usually a gradual increase. This high initial rise increased with rising wind velocity, being 78 per cent. for 2.6 m.p.h., 89 for 5.4, 98 for 9.5, and 138 for 16 m.p.h. These values are considerably higher than the averages for the entire periods that the wind was acting. These latter values are given in table II, for both the day and night periods, and the daytime ones are shown graphically in figure 3. The

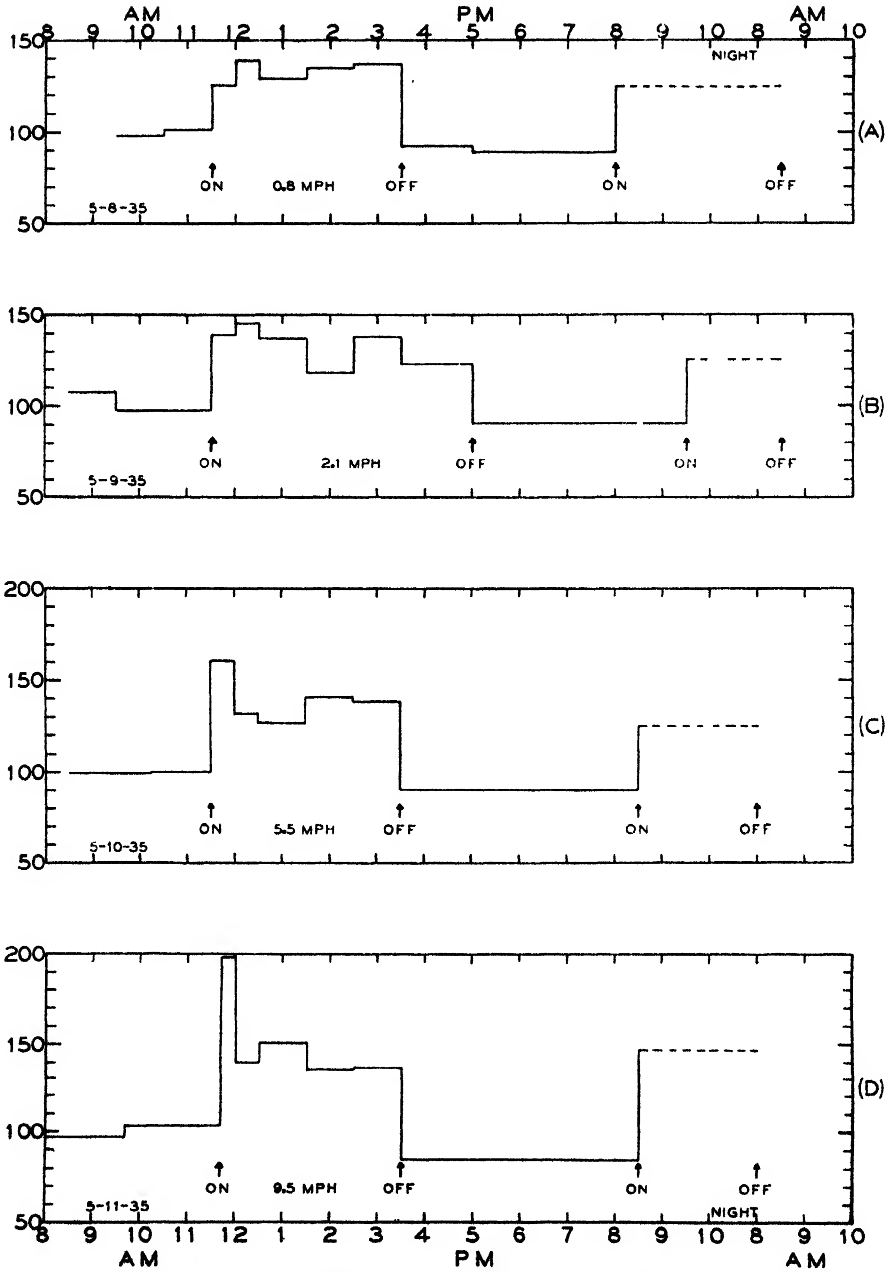

FIG. 1. Results from four of the short time series taken inside the greenhouse. The ordinate represents the transpiration rate of the test set in percentage of that of the control, while the abscissa indicates time of day. Values obtained during the night are shown by the dashed lines.

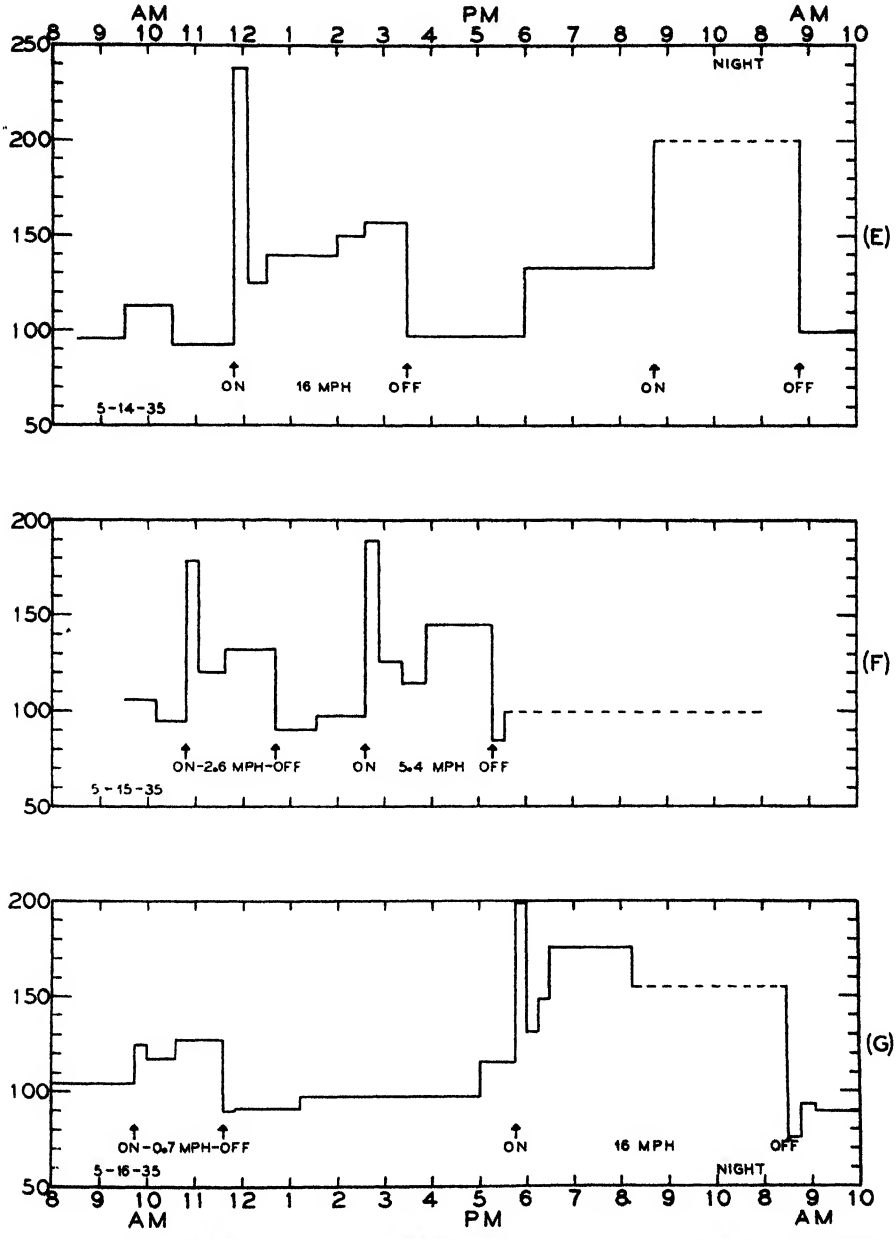

FIG. 2. Results from three others of the short time series. Ordinates are the same as in figure 1.

TABLE II

General summary of results from series taken inside greenhouse

Date	Daytime interval	Wind velocity	Av. T_b/T_a	Nighttime interval	Av. T_b/T_a
		m.p.h.			
5/16/35	9:44 A.M.–11:34 A.M.	0.7	1.23	.	..
5/ 8/35	11:30 A.M.– 3:30 P.M.	0.8	1.32	8:00 P.M.–8:30 A.M.	1.24
5/ 9/35	11:30 A.M.– 5:00 P.M.	2.1	1.34	9:30 P.M.–8:50 A.M.	1.26
5/15/35	10:48 A.M.–12:41 P.M.	2.6	1.34	.. . .	
5/15/35	2:38 P.M.– 5:18 P.M.	5.4	1.40	.. . .	...
5/10/35	11:30 A.M.– 3:30 P.M.	5.5	1.38	8:30 P.M.–8:00 A.M.	1.25
5/11/35	11:40 A.M.– 3:30 P.M.	9.6	1.46	8:30 P.M.–7:30 A.M.	1.47
5/14/35	11:50 A.M.– 3:30 P.M.	16	1.50	8:45 P.M.–8:50 A.M.	2.00
5/16/35	5:45 P.M.– 8:15 P.M.	16	1.71	8:15 P.M.–8:28 A.M.	1.54

ratio of T_b to T_a rises rapidly with velocity up to about 2 m.p.h., but the increase thereafter is much slower. A wind of 1 m.p.h. causes a rise in the transpiration rate of about 30 per cent., while one of 16 m.p.h. induces only about 50 per cent. The night values are of approximately this same order of magnitude, although the low velocities appear to have less effect and the

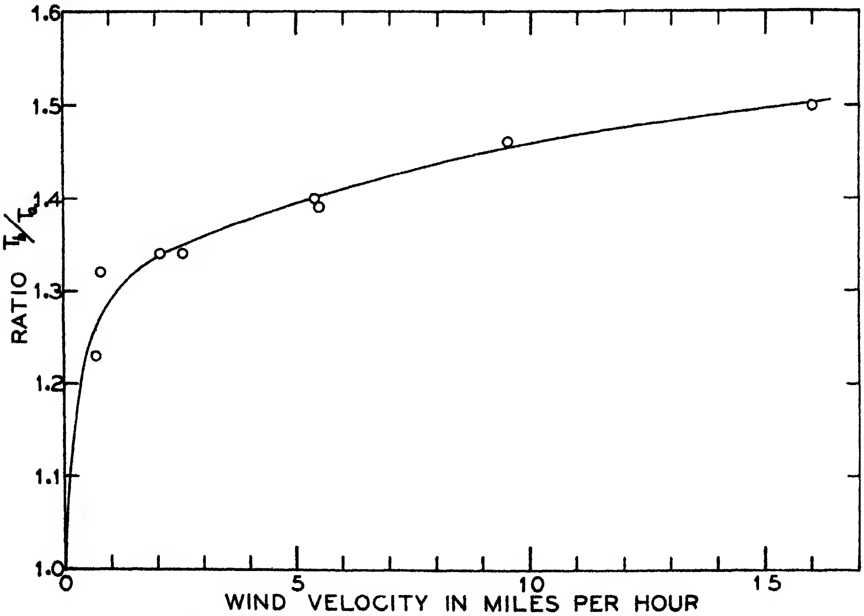

Fig. 3. The ratio of the transpiration rate of the test set (T_b) to that of the control (T_a) represented as a function of wind velocity. The values are all averages for from 2 to 4 hours of wind during the daytime hours.

high ones more effect than during the day. Although the *relative* increase in transpiration rate during the night is nearly the same as during the day, the *actual* increase in grams per square decimeter per hour is much less. This effect is shown graphically in figure 4 by the data taken May 11, 1935.

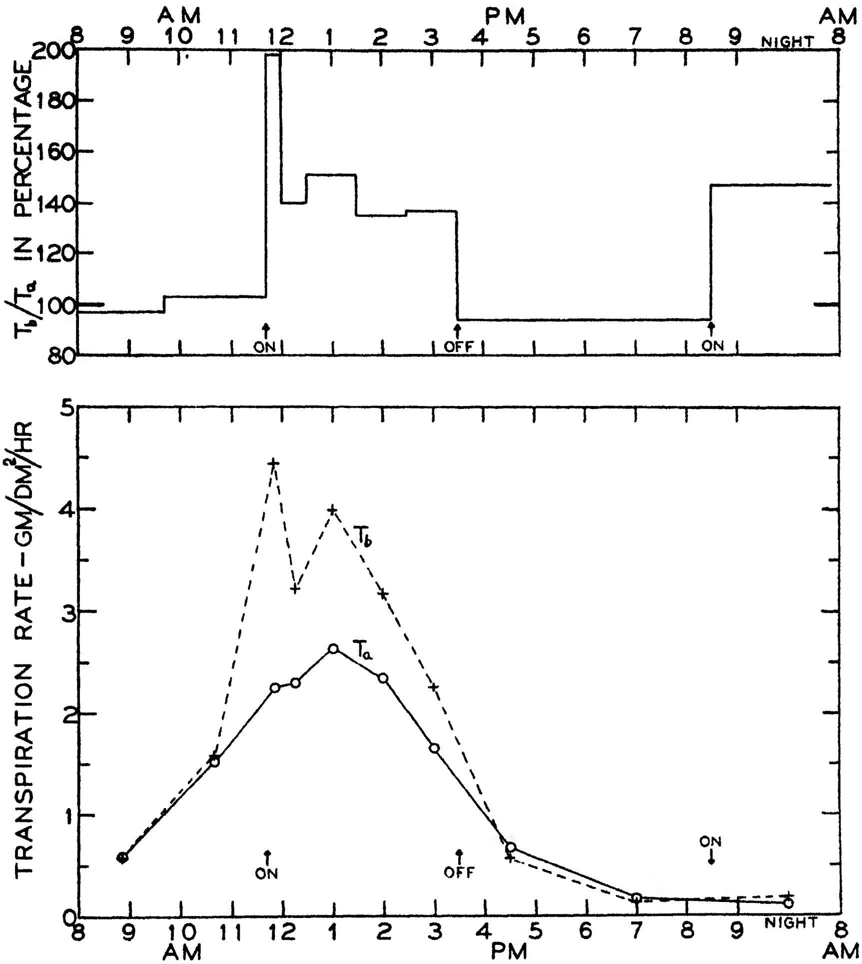

FIG. 4. Results from the short time series of May 11, 1935, with a wind velocity of 9.5 miles per hour. The actual transpiration rates of the test and control sets are shown in the lower part as T_b and T_a respectively, while their ratio in percentage is shown above.

The lower section of figure 4 shows the actual transpiration rates of the two sets of plants and the upper their ratio. Wind in this case caused a rise of about 46 per cent. both day and night, although the actual increase in grams per square decimeter per hour during the day was about 20 times as great as during the night.

The behavior of the stomata during the various series depended on the wind velocity, currents below about 2 m.p.h. having no observable effect, those above this value usually showing a distinct influence. The stomatal openings for some of the series, given in table III, show the action typical

TABLE III

STOMATAL OPENINGS FOR SOME OF THE SERIES TAKEN INSIDE THE GREENHOUSE

5/10/35			5/11/35			5/15/35			5/16/35		
TIME	*a*	*b*	TIME	*a*	*b*	TIME	*a*	*b*	TIME	*a*	*b*
8:30 A.M.	5	5	8:00 A.M.	5	5	10:50 A.M.	5	5	5:45 P.M.	3	3
10:30	5	5	9:45	5	5	Wind of 2.6			Wind of 16		
11:30	5	5	11:30	5	5	m.p.h on at			m.p.h. on at		
Wind of 5.5			Wind of 9.5			10:50 A.M.			5:45 P.M.		
m.p.h on at			m.p.h. on at			11:10	5	1	6:00	3	1
11:30 A.M.			11:40 A.M.			11:45	5	2	6:15	3	1
12:00	5	1	12:00	5	0	Wind off at			6:30	3	1
12:30 P.M.	5	1	12:30 P.M.	5	1	12:42			8:20	0	0
1:30	4	1	1:30	5	1	12:45 P.M.	5	2	8:30 A.M.	5	3
2:30	4	1	2:30	5	1	2:35	5	4	Wind off at 8:30		
3:30	4	1	3:30	5	1	Wind of 5.4			10:15 A.M.	5	5
Wind off at 3:30			Wind off at 3:30			m.p.h. on at					
5:15	1	3	5:30	2	1	2:38 P.M.					
8:15 P.M.	0	1	8:30 P.M.	0	0	3:00	5	1			
			Wind of 9.5			3:30	5	1			
			m.p.h. on at			4:00	4	1			
			8:30 P.M.			5:20	3	2			
			7:30 A.M.	4	2	Wind off at 5:20					
						8:00 A.M.	5	5			

*Stomatal openings are graded according to an arbitrary scale with 0 denoting closed and 5 wide open; *b* is the test set and *a* the control.

of the effect of wind. The stomata closed, although usually not completely, in less than half an hour and in some cases in less than 15 minutes, and remained so the rest of the day, provided the wind was blowing. However, if the wind ceased, the stomata usually reopened within a couple of hours. When the wind blew all night, the stomata opened the next morning to only about half their maximum. (For other pertinent data see table IV.)

The high initial rates of transpiration at the onset of wind of the higher velocities caused a certain amount of wilting, as evidenced by the flaccid appearance of the leaves at the end of the first period after the wind was turned on. This effect was evident in the series of May 15 with the 5.4 m.p.h. wind, that of May 11 with the 9.5 m.p.h. wind, and those of May 14 and 16 with the 16 m.p.h. wind, but in none of the others. In all cases this effect appeared in about 15 minutes and vanished in about half an hour, and in no case was it very marked. Wind of 16 m.p.h. caused slight bruising around the edges of some of the leaves and in places where they folded over or touched each other; however, this effect was not noticeable with lower velocities.

TABLE IV

VALUES OF AIR TEMPERATURE IN DEGREES FAHRENHEIT AND RELATIVE HUMIDITY IN PERCENTAGE FOR THE SERIES TAKEN INSIDE GREENHOUSE

5/8/35	Time	9:30	11:30	12:30	1:30	2:30	3:30	5:15	8:20	
	Air temp.	74	75	83	86	91	87	78	67	
	Rel. hum.	58	51	47	47	40	41	47	56	
5/9	Time	8:30	9:30	11:30	12:30	1:30	2:30	3:30	5:15	9:30 P.M.
	Air temp.	72	76	77	82	80	77	77	72	65
	Rel. hum.	59	53	54	50	52	52	52	57	68
5/10	Time	8:30	10:15	11:30	12:30	1:30	2:45	3:30	5:15	8:30
	Air temp.	65	73	82	80	76	81	83	78	62
	Rel. hum.	68	57	51	52	55	51	50	53	64
5/11	Time	9:45	11:45	12:30	1:30	1:55	2:30	3:30	5:30	8:30
	Air temp.	84	99	102	108	100	95	84	72	63
	Rel. hum.	51	36	36	33	37	30	37	43	57
5/14	Time	8:30	9:30	10:30	12:00	12:30	2:00	3:30	6:15	8:45
	Air temp.	69	70	78	80	81	81	78	70	61
	Rel. hum.	59	55	46	45	47	47	49	53	63
5/15	Time	9:00	10:20	11:15	12:45	1:45	2:45	3:45	5:30	
	Air temp.	74	80	81	78	86	84	81	84	
	Rel. hum.	58	45	49	51	44	43	44	46	
5/16	Time	8:00	10:00	11:30	12:00	87	86	80	64	80
	Air temp.	72	85	102	95	37	33	39	58	47
	Rel. hum.	55	41	36	31	1:20	5:20	6:05	8:20	8:30 A.M.

The very high initial rate of transpiration that takes place at the onset of wind in the higher range of velocities apparently causes a reduction in the sap content of the leaves as shown by their slightly flaccid appearance at this time and a consequent closure of stomata, both of which factors doubtless play a part in the reduction of the relative transpiration rate that occurs during the second weighing interval. Following this second period, the flaccidity disappears and there usually occurs a rather slow increase in the relative transpiration rate, which continues until the wind ceases. The recovery of turgor in the leaves and the fact that the stomata remain at about the same degree of opening during this period of gradual increase indicate that the rise must be due to a gradual restoration of the sap content of the leaves with resultant increase of transpiration. The fact that this gradual increase exists at all indicates that the closure of the stomata retards transpiration sufficiently to permit recovery of the sap content of the leaves.

On the other hand, an indication that the stomata do not always play a controlling part in regulating transpiration is obtained from the relative transpiration rate immediately after the wind stops. Though there is at this time practically always a reduction in the rate of the test set below that of the control, it is rarely more than 20 per cent., in spite of the fact that the stomatal opening may be considerably less. In this connection it is important to note that even in cases when the stomata are open, a drop of about 10 per cent. occurs, due probably in these cases to the lower sap content of the leaves. This view is supported by the fact that the relative influence of wind on the rate of water loss during the night, when the stomata are closed, is much the same as during the day. This means either that wind has practically the same effect on the cuticular as on the stomatal component of transpiration, or that stomata may sometimes play only a small part in the regulation of transpiration. This latter view is supported by the work of other investigators (**10, 13, 15, 16**).

Comparison of the data from different series indicates that the closure of stomata by wind is not entirely due to the water relations, but at least partly to mechanical action. This is well illustrated by comparison of the stomatal behavior of the test set on May 10 (wind of 5.5 m.p.h.) with that of the control on May 11. In the former case, the stomata closed and remained so while the wind continued, but in the latter they remained wide open, even though the transpiration rate throughout the series was nearly twice as great. A direct test of this assumption is difficult, since it is hard to shake a leaf without increasing its transpiration, and it is also difficult to prevent wind shaking the leaf. This effect is in agreement with the work of KNIGHT (**11**) and LOFTFIELD (**16**), who found that the stomata of some plants are sensitive to the shock of handling the leaves.

Discussion

The assumption that the high initial rise in the transpiration rate that occurs at the onset of winds of velocities greater than about 3 m.p.h. lasts probably less than 15 minutes is supported by the experimental data. The effect is illustrated best by the series of May 16 with the 16 m.p.h. wind. In this series, the transpiration rate for the first 15 minutes after the wind started was 2.08 grams per square decimeter per hour, representing an increase of 100 per cent. with respect to the control, while for the next period, also 15 minutes, the rate was 0.92, or an increase of only 31 per cent. with respect to the control. This large decrease in the relative transpiration rate during the second period indicates that at the beginning of the initial period the increase in the rate of the test set must have been much greater than 100 per cent. and at the end smaller. This assumption is further supported by the fact that at the end of the initial 15-minute period, the leaves showed some evidence of flaccidity even though the transpiration rate was only 2.08 gm./dm.^{2}/hr., while during the period 12:30–1:30 on May 11 the rate of transpiration of the test set was 3.98 with no evidence of flaccidity. This sort of action appears to be explainable only by the assumption that the transpiration rate must be very high for the first few minutes of the initial period of wind with a subsequent rapid decrease. It is unfortunate that the methods used in these experiments did not permit a direct test of this assumption, but 15 minutes was the shortest time interval that could be employed without too great sacrifice of accuracy in weighings.

When the stomata do respond to the action of wind, they close partially or completely, as the case may be, in less than half an hour, and for the high velocities in less than 15 minutes. The lowest velocity of wind that will induce closure of stomata seems to be somewhere between 2 and 3 m.p.h., although some series have been taken in which wind of as high as 5 m.p.h. had no noticeable effect on the stomata. In these, however, there was no indication of the very high initial rise in the relative transpiration rate that occurs in the series given here with the same wind velocity, although the average increase in the rate for the entire period of wind agreed very closely. The range between 2 and 5 m.p.h. appears to be critical in that wind of these velocities will sometimes close stomata, sometimes not, apparently depending upon some factor or factors to which it cannot at present be assigned. Below this range, wind has not been observed to affect the stomata, but above it invariably does so.

II. Effect of wind on growth and transpiration; long time series

Methods

For these experiments, plants of *Helianthus annuus* were grown from seed in cans of the type described above, with three or four seeds in each.

When the plants had reached the first leaf stage, they were selected for uniformity of height and leaf size, leaving only one in each can. The cans were then sealed with sand and cotton, and placed in wooden boxes $2 \times 4 \times 1$ ft. deep, sunk in the ground with their tops level with the surface. These boxes were covered with rather closely fitting lids of masonite in order to prevent the large variations in soil temperature that would occur if the cans were exposed directly to the sun's rays. Four of these boxes were used with six phytometers and one check can in each. One set served as a control with only the natural wind, while the other three had artificial wind of about 5, 10, and 15 miles per hour produced by the fans described above. These fans measure approximately 20 to 22 inches in diameter, so that the stream of air was quite wide enough to cover the entire set of plants throughout the growing period.

The water content was maintained constant within a few per cent. and a record of water used was kept. Measurements of leaf areas and diurnal variations in transpiration rates and stomatal openings were made at intervals during the growing period. When the plants had become as large as the wind current would cover, the series was terminated, final measurements of leaves and stems were made, and the plants were dried in a large oven to obtain their dry weights (**17**).

Of the four series conducted, two were in the fall and two in the spring. Series I began with the planting on September 25, 1933, and ended November 17; the second ran from March 13, 1934, to May 2; the third started September 15, 1934, and ended October 26; and the fourth lasted from February 26, 1935, to April 25.

Results

The average leaf areas of plants taken at intervals during series I are given in table V and are shown graphically in figure 5. The four curves, A, B, C, and D, correspond to the four sets of plants, A, B, C, and D, respec-

TABLE V

AVERAGE LEAF AREAS OF PLANTS IN SQUARE DECIMETERS AT INTERVALS DURING THE GROWING PERIOD; SERIES I

	Date					
A	1.16	3.58	10.70	19.00	22.20	46.5
B	1.17	3.78	10.20	18.30	21.20	44.5
C	0.83	2.20	5.50	10.90	12.90	26.8
D	0.70	1.92	3.63	6.33	7.33	13.9

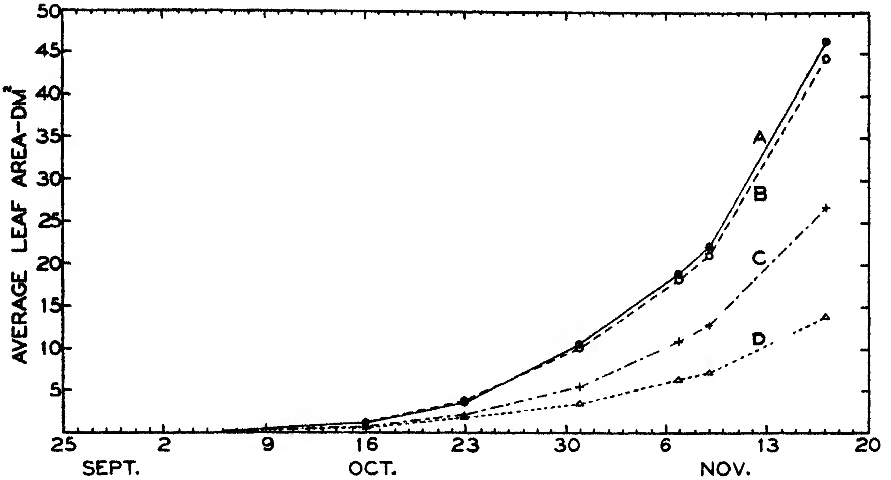

FIG. 5. Average leaf areas in square decimeters for the four sets of plants A, B, C, and D with wind velocities of about 0, 5, 10, and 15 miles per hour respectively, determined at intervals during series I.

TABLE VI

WEEKLY AVERAGE TRANSPIRATION RATES DURING THE GROWING PERIOD FOR SERIES IV

PERIOD	SET NO.	AVERAGE TRANSPIRATION RATE	
		GM./DM.²/24 HR.	PERCENTAGE OF CONTROL
3/20/35	A (control)	11.4	100
to	B	11.4	100
3/27	C	11.0	96
	D	10.9	96
3/27	A	5.94	100
to	B	6.40	108
4/3	C	7.09	119
	D	7.88	133
4/3	A	8.04	100
to	B	9.62	120
4/10	C	10.8	134
	D	10.3	128
4/10	A	11.3	100
to	B	14.0	124
4/17	C	15.9	141
	D	17.5	155
4/17	A	12.6	100
to	B	15.0	119
4/24	C	16.8	133
	D	18.3	145

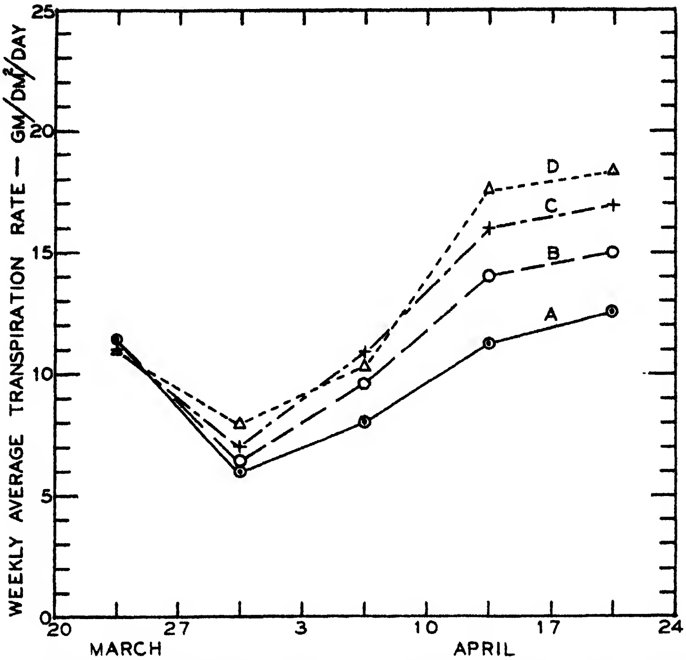

FIG. 6. Average transpiration rates for weekly periods during series IV.

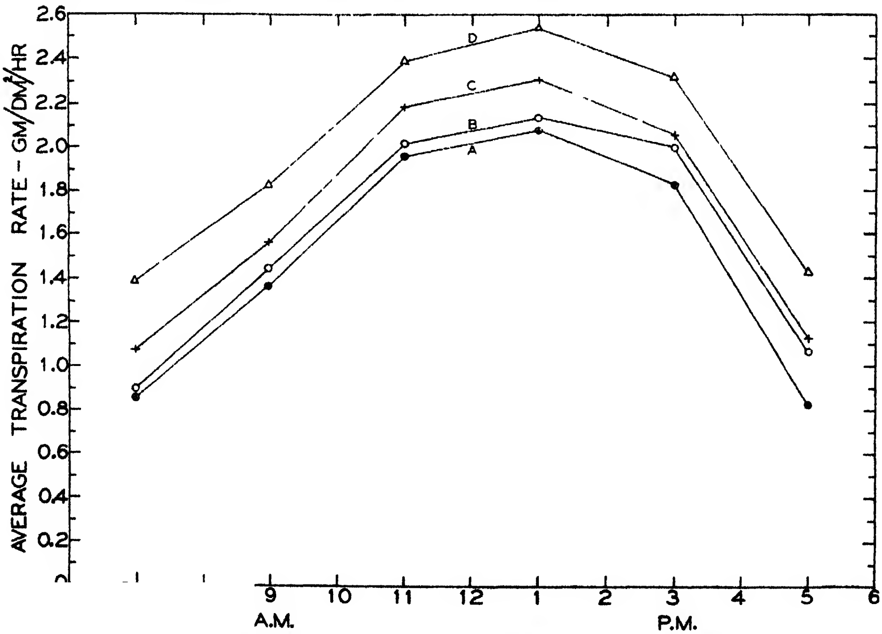

FIG. 7. Daily march of transpiration of the four sets of plants near the end of series II. Data taken May 2, 1934, at which time the plants were 7 weeks of age.

TABLE VII

DAILY MARCH OF TRANSPIRATION OF PLANTS GROWN UNDER CONTINUOUS WIND; SERIES II. DATA TAKEN MAY 2, 1934, AT WHICH TIME THE PLANTS WERE 7 WEEKS OF AGE. VALUES GIVEN ARE IN GRAMS PER SQUARE DECIMETER PER HOUR

SET NO.	6–8	8–10	10–12	12–2	2–4	4–6	6–6 (NIGHT)
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A	0.86	1.37	1.96	2.08	1.83	0.83	0.071
B .	0.90	1.45	2.02	2.14	2.00	1.07	0.005
C	1.08	1.57	2.10	2.31	2.06	1.13	0.191
D	1.39	1.83	2.39	2.54	2.32	1.43	0.306

tively, with wind velocities of approximately 0, 5, 10, and 15 miles per hour. The average transpiration rates for weekly periods during series IV were obtained from the average leaf areas and total transpiration during those periods; they are given in table VI and are shown graphically in figure 6.

TABLE VIII

RESULTS FROM THE FOUR SERIES

SERIES	SET NO.	AV. LEAF AREA	AV. STEM HT.	AV. STEM DIAM.	AV. DRY WT.	AV. TOTAL TRANSPI-RATION	AV. WATER REQUIRE-MENT	AGE OF PLANTS	NO. PLANTS PER SET
		dm.^{2}	*cm.*	*mm.*	*gm.*	*gm.*	*gm./gm.*	*days*	
	A	46.5	62	14.7	33.5	7520	224		5
I	B	44.5	63	15.0	29.0	6870	237	53	6
	C	26.8	40	11.4	18.5	4960	268		6
	D	13.9	33	10.3	11.2	3210	286		6
	A	28.0	63	13.3	40.0				6
II	B	18.7	47	12.2	28.6			50	4
	C	18.0	45	11.7	26.6				5
	D	13.5	44	11.6	22.4				6
	A	37.0	53	13.9	21.3	4730	222		6
III	B	31.8	42	12.5	19.1	4320	226	41	5
	C	15.5	33	9.8	10.2	2770	271		6
	D	8.5	23	8.6	6.2	1760	284		6
	A	15.4	48	11.3	16.5	2640	160		6
IV	B	14.0	48	11.2	15.8	2765	175	58	6
	C	12.9	47	11.0	12.2	2515	206		6
	D	9.3	32	9.6	7.1	1770	249		5

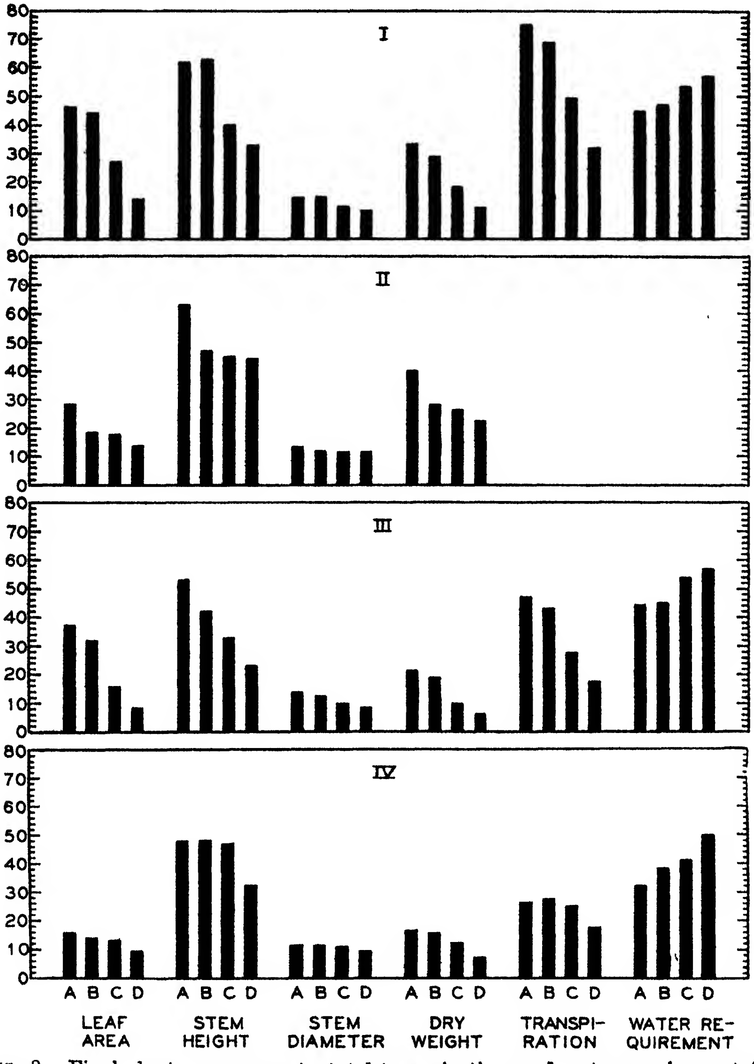

FIG. 8. Final plant measurements, total transpiration, and water requirement for all series. The ordinate represents leaf areas in square decimeters, stem heights in centimeters, stem diameters in millimeters, and dry weight in grams, but only 1/100 of the total transpiration in grams and 1/5 of the water requirement in grams of water per gram dry weight of material produced.

For the first week after subjection to wind, sets A and B had the same transpiration rate, while sets C and D were a little less. Thereafter, however, the transpiration rates per unit area increased with the wind, reaching a maximum for the highest velocities during the last two weeks of the series of from 35 to 50 per cent. more than the control.

The daily march of transpiration near the end of the series is represented by the data from series II given in table VII and shown graphically in figure 7. The transpiration rate at all times of day increased with wind velocity, but the influence is relatively greater at night than during the day. For example, set D (15 m.p.h. wind) at night used water from four to five times as rapidly as the control, while during the day only about 1.4 times. Measurements of stomatal openings during such series showed that for the greater part of the first two weeks the stomata were kept partially closed by the two highest winds, but thereafter wind had no noticeable effect on their diurnal cycle.

The final plant measurements from all four series are given in table VIII and are shown graphically in figure 8. In all series, leaf area, stem height and diameter, dry weight, and total transpiration (with one exception) decreased with increasing wind velocity. Inversely, the water requirement increased, the maximum increment observed being about 50 per cent. in the case of the 15 m.p.h. wind in series IV. The total transpiration and water requirement for series II could not be obtained on account of an unexpected rain that permitted unknown amounts of water to enter the cans.

All series were qualitatively alike in that wind always caused a reduction in growth, although quantitatively there were differences, the greatest being that the effect of wind in the fall seemed to be a little greater than in the spring. In the fall, the 15 m.p.h. wind caused a reduction in dry weight to about one third that of the control, but in the spring to only about one half. Some of the observed differences are doubtless due to statistical fluctuations caused by individual differences, but some are probably caused by diversity in

TABLE IX

WEEKLY AVERAGES OF AIR TEMPERATURE (T) IN DEGREES FAHRENHEIT, AND RELATIVE HUMIDITY (RH) IN PERCENTAGE FOR THE FOUR SERIES

SERIES I			SERIES II			SERIES III			SERIES IV		
WEEK ENDING	T	RH	WEEK ENDING	T	RH	WEEK ENDING	T	RH	WEEK ENDING	T	RH
	°F.	%		°F.	%		°F.	%		°F.	%
10/9/33	62	83	3/19/34	57	87	10/1/34	69	44	3/4/35	51	78
10/16	60	68	3/26	58	86	10/8	62	83	3/11	48	76
10/23	64	53	4/2	64	68	10/15	61	83	3/18	55	75
10/30	57	91	4/9	59	89	10/22	58	89	3/25	48	74
11/6	60	70	4/16	57	85	10/29	60	78	4/1	53	89
11/13	61	52	4/23	58	84				4/8	56	88
11/20	57	77	4/30	65	71				4/15	54	86
			5/7	64	69				4/22	57	87
									4/29	59	80

other environmental factors, thus altering the effect of wind. Weekly averages of air temperature and relative humidity during the four series are given in table IX.

The response of an average plant from each of the four sets in series III, arranged in order of increasing wind velocity, is shown by figure 9. Winds of 10 and 15 m.p.h. caused the well known gnarled and stunted appearance that is characteristic of trees growing in windy habitats. The leaves were rougher, fewer, more wrinkled, and somewhat less fully expanded than those of the control. The number of stomata per unit area rose with increasing wind velocity and the corresponding failure of the leaf to expand, a count for series IV yielding 188, 199, 260, and 304 per square millimeter for sets A, B, C, and D respectively. Microscopic sections of the leaves,

FIG. 9. Typical plants from the four sets in series III. Sets A, B, C, and D, with wind velocities of about 0, 5, 10, and 15 miles per hour respectively, are represented in order from left to right.

however, failed to show any material differences in the mesophyll, owing evidently to compensation by change of size. The stems, on the other hand, showed considerable divergence, particularly in the xylem elements. Camera lucida drawings of quadrants of the four stems, with only the xylem and bast strands outlined, are shown in figure 10. Stems of plants grown under the 15 m.p.h. wind (set D) showed 22 members of the first rank of bundles and only 6 of the second, while those of the control (set A) exhibited 22 of the first, 22 of the second, and 7 of the third. The intermediate sets fell between these two extremes. Although the number of members of the first rank of bundles was the same for both sets A and D, the number of vessels in each bundle was quite different. Each first rank bundle of A was made up of about 50 vessels while those of the same rank in D had only 30. Similarly, the area occupied by the xylem was considerably larger in A than in D, the former being 3.87 mm.^{2}, the latter 1.20. The cross-section area of the stems A and D were 113.0 and 36.6 mm.^{2}, respec-

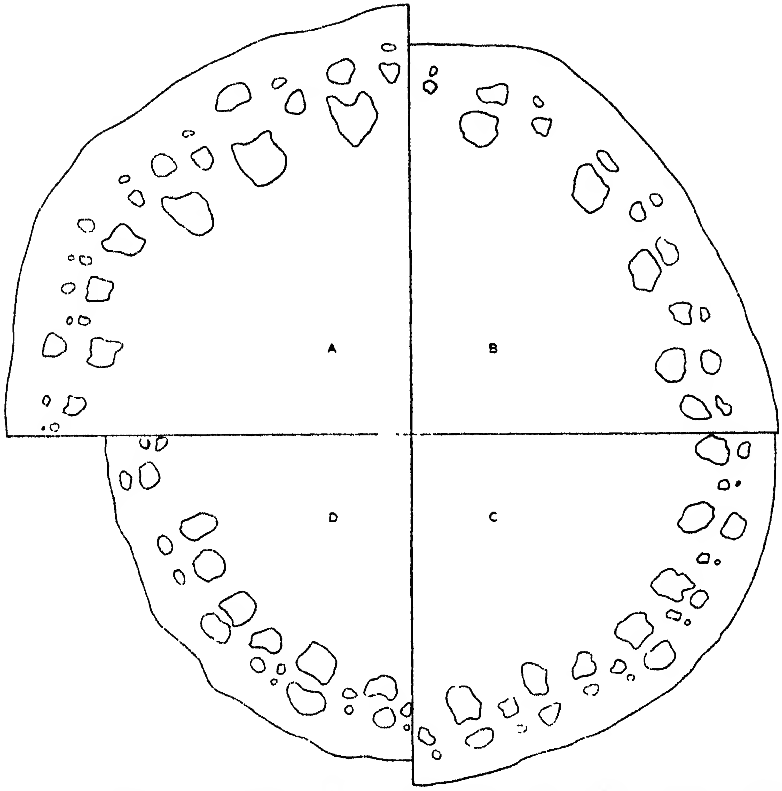

FIG. 10. Camera lucida drawings of quadrants of stems from typical plants from each of the four sets in series IV, with only the xylem and bast strands outlined.

tively; hence the proportion of the total stem area occupied by the xylem was practically the same in both cases, being 3.4 per cent. in the former and 3.3 in the latter.

Summary

PART I

EFFECT OF WIND ON TRANSPIRATION; SHORT TIME SERIES

1. The effect of wind on the transpiration rate of *Helianthus annuus* depends on the velocity. With velocities up to about 2 m.p.h., the transpiration rate increases at the onset of wind about 20 to 30 per cent. and maintains this value as long as the wind acts. For velocities above this, however, there is usually a rather high relative increase for the first few minutes after the wind is turned on, followed by a fall, which in turn is followed by a gradual increase. The initial increase in the rate of water loss rises with increasing velocity, reaching 138 per cent. in the case of the 16 m.p.h. wind. This causes

slight wilting of the leaves and closure of the stomata with consequent reduction in the transpiration rate. The leaves usually recover their turgidity within half an hour or so after the stomata close, and the transpiration rate rises slightly.

2. The average increase of the transpiration rate over a period of 2 to 4 hours rises very rapidly with velocity up to about 2 or 3 m.p.h., but the curve flattens out in this range and mounts only gradually thereafter. Wind of 1 m.p.h. causes a rise of about 30 per cent., while one of 16 m.p.h. induces about 50 per cent.

3. Wind apparently has the same relative effect at night as during the day, causing approximately the same percentage increase in the transpiration rate in both cases.

4. Evidence is presented which indicates that the closure of stomata by wind is partly mechanical and partly due to lowering the sap content of the leaves. Winds above 5 m.p.h. practically always induce closure of the stomata and below 2 m.p.h. almost never, while between 2 and 5 m.p.h., only part of the time.

5. The data also indicate that stomata may close to about one fifth of their maximum opening without affecting the transpiration rate markedly.

Part II

Effect of wind on growth and transpiration; long time series

1. Plants of *Helianthus annuus* have been grown under continuous winds of about 5, 10, and 15 m.p.h. from the first leaf stage to about 6 to 8 weeks of age, using large automobile fans driven by electric motors. Four series were made, two in the spring, two in the fall.

2. For the first week the transpiration rates of all three test sets were practically the same as that of the control, but thereafter the rate began to increase with increasing wind velocity so that by the end of the series the three test sets were using water at rates about 20, 35, and 50 per cent. higher per unit area of leaf surface than the control.

3. In all series, leaf area, stem height and diameter, dry weight, and total transpiration decreased with increasing wind velocity. The water requirement increased, the maximum increment observed being about 50 per cent. in the case of the 15 m.p.h. wind in series IV.

4. The depressing effect of wind on growth appears to be somewhat greater in the fall than in the spring. In the former cases, wind of about 15 m.p.h. caused a reduction in dry weight to about one third that of the control, but in the spring to only approximately one half.

5. In these series, the effect of wind on the transpiration rate is relatively considerably greater during the night than during the day. During

the day, the plants under the 15 m.p.h. wind transpired about 1.5 times as rapidly per unit area as the control plants, while during the night they transpired from 4 to 5 times as rapidly. However, the actual increase in grams of water is much less during the night.

6. Microscopic sections of the leaves of plants from the various sets failed to show any material differences in the mesophyll, due evidently to compensation by change of size. The stems, however, showed considerable difference, especially in the xylem elements. Stems of plants grown under the 15 m.p.h. wind showed 22 members of the first rank of bundles and only 6 of the second, while those of the control set exhibited 22 of the first, 22 of the second, and 7 of the third. Similarly, the area occupied by the xylem elements decreased with increasing wind velocity. However, the total cross-section area of the stems decreased in the same ratio so that the proportion of the total stem area occupied by the xylem showed no significant difference.

7. The plants grown under the higher wind velocities showed the gnarled and twisted appearance characteristic of trees growing in windy habitats.

8. Wind is to be regarded as a factor, though not the paramount one, in the dwarfing characteristic of the alpine tundra and in the reduced and procumbent forms of coastal dunes, as exemplified in the experimental areas on Pike's Peak and the dune gardens at Santa Barbara.

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FURTHER STUDIES OF ELONGATION AND EXPANSION IN *HELIANTHUS* PHYTOMETERS

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(WITH NINE FIGURES)

Introduction

The investigation of the factors and functions involved in elongation and expansion under reduced light intensity has been continued with the special purpose of relating these to structural modifications as well as to growth forms. The ultimate objectives in these studies are to measure in terms of adaptation the climates and edaphic habitats characteristic of the various transplant gardens and to permit interpreting the functional responses of natives not susceptible of phytometer treatment. At the same time, opportunity was taken to increase the number of light values to six, in order to simulate the whole range of natural habitats, as well as the control conditions in the lath-houses. In addition, an inquiry was made into the effect of size of container, and a battery of free phytometers was installed in the soil under each light intensity in order to integrate all the direct factors of the six eces.

Methods

The several light values were secured by means of lath-huts 6 ft. high and 8×12 in section. These were constructed of standard plaster lath $1\frac{1}{2} \times \frac{3}{8}$ inches in section, which were so spaced as to produce approximately the five intensities desired. The relative spacing and light values were as follows: (1) spacing $4\frac{3}{4}$ in., intensity 65 per cent. of full sunshine; (2) spacing $1\frac{3}{4}$ in., intensity 44 per cent.; (3) $\frac{3}{4}$ in. apart, 27 per cent.; (4) $\frac{3}{8}$ in. apart, 19 per cent.; (5) $\frac{3}{16}$ in. apart, 11 per cent. These values were approximately the same as in the large lath-houses used for producing adaptations, but the lowest of these had a rating of but 6 per cent., thus yielding six conditions beside full sunshine. Each hut was provided with a door of the same construction and after serious experience with on-shore winds, was firmly anchored at the four corners. Provision was also made for raising the huts several feet to accommodate height growth in the higher intensities. To facilitate weighing the exceptionally large number of phytometers, a hut 12 ft. tall and 6 ft. in cross section was built nearby and this served also for photographic purposes.

A stopwatch photometer was employed to measure intensities, and readings were taken hourly in the sun and in each hut for representative days during the growing season. For the exposure, the photometer was carried

through a wide circle with a uniform motion in order to integrate as accurately as possible the alternating bands of sun and shade. While this procedure does not insure exact results, it is the best so far devised for lath-house conditions. The many exposures were then evaluated by the usual standard and graphs were made for the intensity through the day for the various habitats, using time of day for the abscissa and percentage of light as the ordinate. By using a planimeter to integrate under the curves, the the respective values were obtained in terms of full sunlight as 100 per cent. From the averages of these were obtained the final values for each hut, these being the mean of the total radiation for the day.

Four sizes of containers were employed, the largest being garbage cans 25 in. high and 17 in. in diameter; the others were respectively $11 \times 8\frac{1}{2}$ in., 10×8 in., and 7×6 in. These three were of the more recent type with conical lids and apertures 2 in. across to accommodate stem and watering tube. The cans were sealed with a mulch of fine dry sand and above this the opening was closed by a band of non-absorbent cotton wrapped about the plant stem. As a special provision against sudden rainstorms, rubber aprons were cut from old inner tubes in the form of circles 12 cm. in diameter, with a hole 1 cm. wide in the middle and a slit running to the margin. When the apron was placed around the stem, the edges of the slit were overlapped and held in place by paper-clips at the outer and inner margins. Contact between rubber and stem was prevented by a thin band of absorbent cotton. The conical shape of the apron led away the rain that might drip down the stem and at the same time prevented injury to the latter, in addition to promoting aeration in some measure. When rains promised to be heavy, canvas covers were placed above the batteries as an additional safeguard.

The soil employed was a good loam of the same quality as in previous experiments and similar technique was used in filling the containers uniformly, as well as in planting the seeds and caring for the seedlings (**3**). The four holards were set respectively at 35, 26, 18, and 14 per cent. of the dry weight of the soil, and six containers of each value were assigned to each of the six light intensities. Five seeds were planted in each container and allowed to germinate, but when the plumules were well developed, one seedling closest to the measured average for the series was selected and the others carefully removed. No further selection was made in organizing the batteries for the various conditions, the cans merely being grouped in accordance with the numerical sequence. All sets were kept in the greenhouse until the seedlings were well established; this period was two weeks for the fall and three weeks for the spring series. As a consequence, some slight differentiation due to holard was apparent at the time the batteries were distributed to the six light intensities.

At the outset, the phytometers were weighed at weekly intervals; but as they grew larger, weighing necessarily became more frequent, first daily and finally three times a day. The amount of water lost was replaced after each weighing in order to maintain the holard as nearly uniform as possible. For this purpose, a 500-cc. burette was employed, the water being delivered to the plant through a tube reaching into the lower part of the container. To avoid any possibility of harmful accumulation of salts from the hard water of the locality, rain water was utilized throughout the series. Measurements of stem height and diameter and of leaf dimensions were taken on the same day and in the same order each week, thus yielding a cumulative record of growth for the entire period. In spite of the fact that the 14 per cent. holard was but 2 to 3 per cent. above the echard for the soil and that the 35 per cent. was practically at the limit for adequate aeration, frequent watering and rate of loss so nearly balanced each other that the tendency toward wilting or an aeration deficit was rarely in evidence. The generally uniform distribution of the water in the soil is reflected in the root systems, which were well distributed and not compacted about the inner surface of the can (fig. 1).

Function and growth responses

Two seasonal installations were employed in this investigation. The fall-winter series was planted October 16, the phytometers distributed to the lath-huts October 30, and the experiment terminated February 11, 1935. The spring series was begun February 28; all cotyledons were expanded by March 8, at which time the various holards were standardized. These batteries were placed in the several situations on March 21 and the final results were secured on June 4. The period of growth for the first series was 119; for the second, 97 days. In both cases the plan had been to follow the course of development to flowering and fruiting, but a heavy gale flattened the lath-huts and abruptly terminated the fall series, destroying the leaves but injuring the heads only slightly. While the spring series suffered no such mishap, the weather conditions of a marine climate and the behavior of such a warm-temperate species as the sunflower precluded maintaining the series until all heads were mature.

The interval between the time of flowering in the sun and in the lowest light intensity was so long that plants in the higher light values lost one-third or more of their leaves. Thus, the average number of leaves produced in the sun and 35 per cent. holard was 37.7 per plant, while by the end an average of but 27 leaves remained, the lower 10 having dried and disintegrated. In 44 per cent. light, the average loss was less, *viz.*, 7 out of 39; in 19 per cent. 3 in 27; and in 11 per cent. 1 out of 22. This inevitably led to a cumulative discrepancy in the photosynthesis and transpiration of

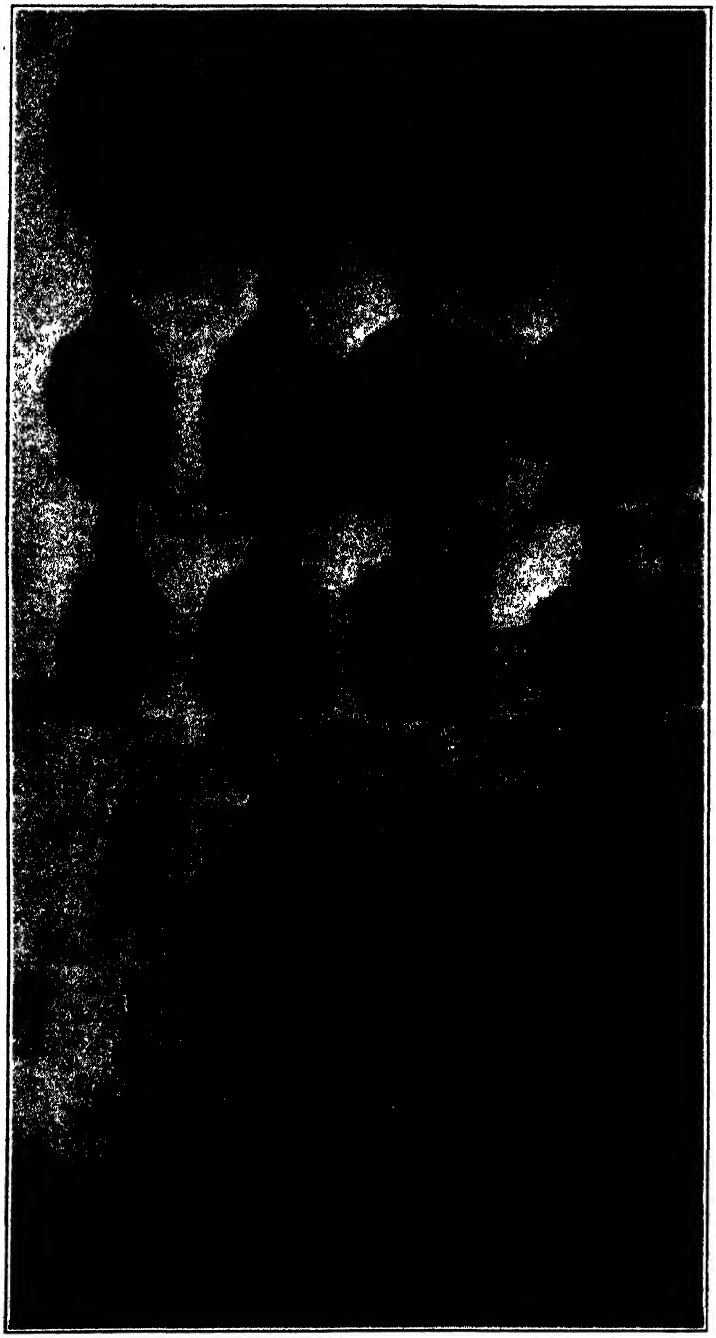

Fig. 1. Series of roots through the four holards and six light conditions.

TABLE I

AVERAGE OF MEASUREMENTS ON JUNE 4 FOR THE LARGE PHYTOMETERS

Factor gradients		Stem				H/D	Leaf area per plant		Leaf number				Transpiration		Weight of plant			Water requirement	
Light	Holard	Height		Diameter					Total		June 4		Total gm. per plant	Total	Wet	Dry	Dry		
%	%	cm.	%	cm.	%		sq. cm.	%		%		%		gm./sq. cm.	gm.	gm.	%	cc./gm.	%
100	35	138.5	100	2.30	100	60.4	5775.0	100.0	37.7	100	26.0	100	18533	3.21	627.1	127.7	100.0	145.3	100
	26	127.9	92	2.04	89	62.5	5180.3	89.8	36.0	95	25.3	97	15194	2.93	508.8	106.8	83.5	142.2	98
	18	111.1	80	1.74	76	63.9	4372.0	75.8	32.2	85	27.7	106	8674	1.83	383.0	67.9	53.1	127.7	88
	14	83.2	60	1.07	47	77.8	2574.0	44.6	26.3	70	22.3	86	4938	1.92	209.8	39.2	30.6	125.9	87
	Ave.	115.2	83	1.79	78	66.1	4475.0	77.6	33.0	88	25.3	97	11835	2.47	432.2	85.4	66.8	135.3	93
65	35	172.0	124	1.85	80	93.0	4626.0	80.1	38.0	101	28.5	110	18448	4.00	507.3	105.4	82.5	175.0	120
	26	135.0	97	1.73	75	78.0	3465.3	60.0	32.2	85	25.3	97	14525	4.20	380.5	79.8	62.4	182.0	125
	18	104.0	75	1.30	57	80.0	3560.3	61.6	29.0	77	22.7	87	9123	2.56	306.1	63.7	49.9	143.2	99
	14	85.2	62	1.10	48	77.5	3022.6	52.4	29.0	77	25.7	99	5322	1.77	230.7	39.9	31.2	133.4	92
	Ave.	124.0	90	1.49	65	82.1	3668.4	63.5	32.1	85	25.5	98	8223	3.14	356.2	72.2	56.5	158.4	109
44	35	191.0	138	2.13	93	89.6	7562.3	131.0	39.0	103	31.7	122	17562	2.32	577.6	105.5	82.5	166.4	115
	26	180.5	130	1.90	83	95.0	6884.0	119.2	31.7	84	25.5	98	13801	2.01	379.0	77.1	60.4	179.0	124
	18	137.0	99	1.40	61	97.9	5080.3	88.0	29.0	77	25.7	99	8529	1.68	308.6	59.1	46.3	144.3	99
	14	107.2	77	1.03	45	104.1	2984.3	51.6	29.3	78	26.0	100	4287	1.43	251.9	30.6	24.0	143.9	99
	Ave.	153.9	111	1.61	71	96.7	5627.7	97.4	32.3	86	27.2	107	11045	1.86	379.3	63.1	53.3	158.4	109

TABLE I.—(*Continued*)

Factor gradients		Stem				H/D	Leaf area per plant		Leaf number				Transpiration		Weight of plant			Water requirement	
Light	Holard	Height		Diameter					Total		June 4		Total gm. per plant	Total	Wet	Dry	Dry		
%	%	cm.	%	cm.	%		sq. cm.	%		%		%		gm./sq. cm.	gm.	gm.	%	cc./gm.	%
27	35	186.0	134	1.71	74	108.8	9645.3	167.0	35.0	93	32.0	123	16435	1.70	499.5	61.8	48.4	265.9	183
	26	192.0	139	1.53	67	125.3	6814.3	117.9	33.0	88	29.7	114	10515	1.55	358.4	57.6	45.1	182.8	126
	18	151.0	109	1.40	61	107.9	4676.3	81.0	30.3	80	28.0	108	6347	1.36	245.6	35.3	27.6	179.8	124
	14	101.7	73	1.03	45	98.6	2901.3	34.7	28.0	74	25.3	97	3672	1.83	143.2	20.6	16.1	178.2	123
	Ave.	157.7	114	1.42	62	110.1	5784.3	100.2	31.6	84	29.0	111	9242	1.61	311.7	43.8	34.3	201.7	139
19	35	200.0	144	1.56	58	128.0	5343.0	92.6	27.6	73	24.6	95	7512	1.41	295.0	24.9	19.5	301.5	208
	26	161.9	117	1.26	55	128.1	3613.1	62.6	27.0	72	25.7	99	4800	1.33	191.2	17.8	13.9	269.6	185
	18	141.0	102	1.00	44	141.0	2628.7	45.5	27.0	72	24.3	93	3538	1.35	133.3	15.9	12.4	222.5	153
	14	102.5	74	.83	36	123.3	1776.6	30.8	25.0	66	22.3	86	2108	1.19	83.5	11.2	8.8	188.2	130
	Ave.	151.3	109	1.16	48	130.1	3440.3	57.9	26.7	71	24.2	94	4490	1.32	175.7	17.5	13.7	245.4	169
11	35	139.0	100	1.13	49	123.0	2279.0	39.4	21.3	82	22.3	59	3018	1.33	96.3	7.5	5.9	402.5	277
	26	130.6	94	1.00	44	130.6	1491.6	25.8	19.6	75	22.3	59	1888	1.27	65.2	5.3	4.2	356.2	245
	18	113.5	82	0.83	36	136.8	1390.5	24.1	21.3	82	21.3	56	1637	1.18	55.8	5.3	4.2	308.8	212
	14	76.0	55	0.70	30	108.6	792.9	13.7	20.0	77	23.6	60	620	.78	37.4	2.9	2.3	213.8	147
	Ave.	114.7	83	0.91	40	124.7	1488.5	25.7	20.5	79	22.4	58	1791	1.14	63.7	5.3	4.1	320.3	220

the several light batteries, and necessitated closing the experiment before the buds opened in the weaker light intensities. In spite of its many advantages, which are distinctly greater than those of any other species so far utilized for standard phytometers, *Helianthus annuus* possesses the two defects of blocking off the lower leaves and of growing slowly in cold seasons such as that of 1934–1935 at Santa Barbara, as also in the climate of the alpine tundra on Pike's Peak.

The results for transpiration and water requirement, as well as for growth in terms of stem, leaf, and dry weight, were in close accord for the two series. The agreement between these and the five series discussed in the previous paper was likewise striking. Since in the present experiment there were six light intensities instead of four, the detailed results are given for light: holard in table I and these are summarized in the next paragraph. The weights were also determined separately for stem, leaves, roots, and heads, as shown in table II. In addition, a comparison is made between the results obtained from the four sizes of containers, not merely in connection with the two series, but also to throw light upon the phytometer method itself.

Under each light intensity, stem height was greatest in 35 per cent. holard and decreased consistently with the reduction to 14 per cent., with a single exception, the maximum of 200 cm. falling at 19 per cent. light and 35 per cent. water content (fig. 2). The greatest average height for the four holards occurred in 27 per cent. light, but 44 and 19 per cent. yielded values only a little less, while the shortest stems were found in sun at 115.2 cm. and 11 per cent. light at 114.7 cm. The diameter of the stem fell off steadily from 35 to 14 per cent. holard in every battery, ranging from the maximum of 2.3 cm. in the sun to 1.13 cm. in 11 per cent. light intensity. The average for each light value decreased from 1.79 cm. in the sun to 0.91 cm. in the deepest shade, that for 65 per cent. light being in disagreement here as in some other places, owing to leakage in a few containers during a heavy rain.

The total leaf area per plant diminished from 35 to 14 per cent. with great regularity, the one exception occurring at 65 per cent. light and 26 per cent. holard. However, the maximum was at 27/35 per cent. with a value of 9645.3 sq. cm. and the minimum of 792.9 at 11/14 per cent., a ratio of approximately 1:12. The highest average was found in 27 per cent. light, followed closely by 44 per cent.; the average area in the sun was three times greater than that for the lowest light intensity. The total number of leaves produced dropped regularly with the holard for the first four light intensities, but was almost constant for the two lower values, the range being from 39 to 21. The average number for each battery fell from 33 in the sun to 22 at 11 per cent. The number of leaves present at the close

TABLE II

Wet and dry weights of plant parts for the spring series

Factor gradients		Wet weight					Dry weight				
Light	Holard	Stem	Leaves	Root	Flower	Total	Stem	Leaves	Root	Flower	Total
%	%	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
100	35	364.5	128.8	125.7	25.8	644.8	83.8	24.4	34.7	5.30	148.20
	26	313.0	145.8	125.7	14.0	598.5	73.7	26.1	19.4	2.73	121.90
	18	207.8	116.5	53.5	5.4	383.2	34.3	19.5	7.8	1.11	62.70
	14	113.0	69.1	32.0	14.2	228.3	18.4	11.6	4.0	2.62	36.60
	Ave.	249.6	115.1	84.2	14.8	463.7	52.5	20.4	16.5	2.94	92.30
65	35	337.8	88.6	70.0	8.8	505.2	74.5	19.4	21.6	2.13	117.63
	26	250.0	66.8	49.2	2.5	368.5	52.7	12.3	12.6	0.60	78.20
	18	100.7	46.8	44.5	70.0	262.0	24.7	10.2	7.7	14.96	57.50
	14	61.7	34.1	29.8	64.1	189.7	11.8	7.1	3.5	11.70	34.10
	Ave.	187.5	59.1	48.4	36.7	331.3	40.9	12.2	11.4	7.35	71.86
44	35	453.9	120.8	79.5	17.5	671.7	83.8	29.1	16.7	3.73	133.33
	26	284.1	124.5	41.2	28.1	477.5	50.9	22.3	9.7	5.70	88.60
	18	132.5	75.5	42.3	54.9	305.2	28.4	15.9	6.4	11.80	64.50
	14	89.7	43.5	25.8	25.7	184.7	15.0	10.8	2.1	5.00	32.90
	Ave.	240.1	91.1	47.2	31.6	409.7	44.5	19.5	9.0	5.43	79.83
27	35	342.3	121.2	51.5	1.2	516.2	36.9	18.1	9.5	0.44	64.94
	26	258.2	100.0	23.6	2.5	384.3	36.8	16.3	7.5	0.66	61.26
	18	154.2	58.5	11.0	8.1	231.8	20.0	10.7	3.1	1.75	35.50
	14	87.0	43.9	9.2	2.1	142.2	10.0	7.0	3.1	0.55	20.65
	Ave.	210.4	80.9	23.8	3.5	318.6	25.9	13.0	5.8	0.60	45.83

TABLE II.—(*Continued*)

Factor Gradients		Wet weight					Dry weight				
Light	Holard	Stem	Leaves	Root	Flower	Total	Stem	Leaves	Root	Flower	Total
%	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
19	35	206.0	50.7	8.3	.0	265.0	16.7	8.5	1.5	0.13	26.83
	26	125.0	28.0	8.0	.0	161.0	11.0	4.4	1.5	0.30	17.20
	18	84.8	18.6	4.3	.0	107.7	8.6	3.2	1.0	0.30	13.10
	14	48.8	19.5	4.4	.6	73.3	5.8	3.3	1.4	0.22	10.72
	Ave.	116.2	29.2	6.3	.6	151.9	8.4	4.9	1.4	0.24	16.96
11	35	79.7	20.0	3.7	0	103.4	4.6	2.6	0.72	0	7.92
	26	50.0	11.0	1.7	0	62.7	3.1	1.9	0.32	0	5.32
	18	38.2	10.2	2.0	0	50.4	2.0	2.4	0.52	0.04	4.95
	14	16.5	6.3	1.0	0	23.8	1.3	1.1	0.30	0.03	3.03
	Ave.	46.1	11.9	2.1	0	60.1	2.7	2.0	0.46	0.04	5.31

was greatest in the intermediate light values, namely, 29 at 27 per cent. and 27 at 44 per cent., and least in 11 per cent. However, the highest average loss of leaves was 8 in the sun, falling to 7, 5, 2.5, and 2 at 11 per cent.

With respect to total transpiration per plant, the descending order was entirely consistent with the reduction in holard. In the first three light

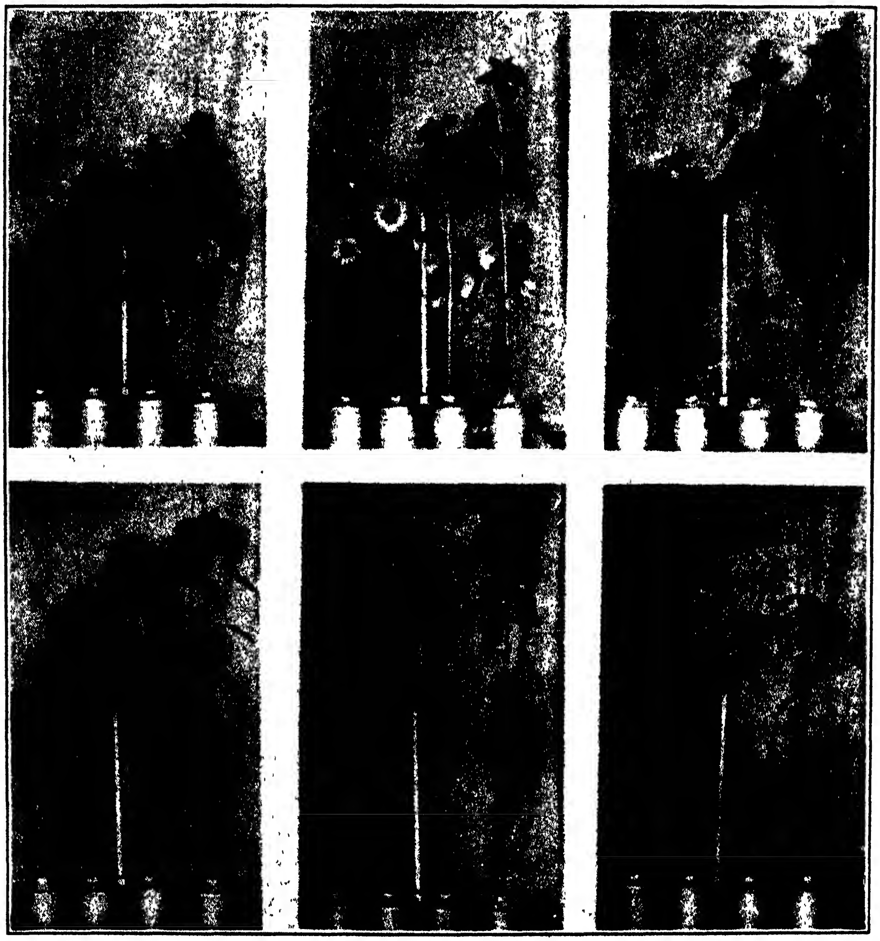

FIG. 2. Representative phytometer sets from each light habitat, arranged according to holards 14, 18, 26, and 35 per cent.

values the loss in 35 per cent. holard was nearly four times as great as in 14 per cent., and in 11 per cent. light, five times as much. The average loss for each light battery was in accord with diminished intensity, except for 65 per cent., as just explained. The loss in grams per sq. cm. decreased with the holard with fair regularity, while it agreed with the light intensity,

with the exception of 65 per cent. again. As a rule the water requirement was highest in the holard of 35 per cent., with two slight exceptions for 26 per cent., and uniformly lowest in 14 per cent. The average water requirement rose consistently with reduced light from 135.3 cc./gm. for sun to 320.3 cc./gm. for the lowest light value, apart from the same figure for 65 and 44 per cent.

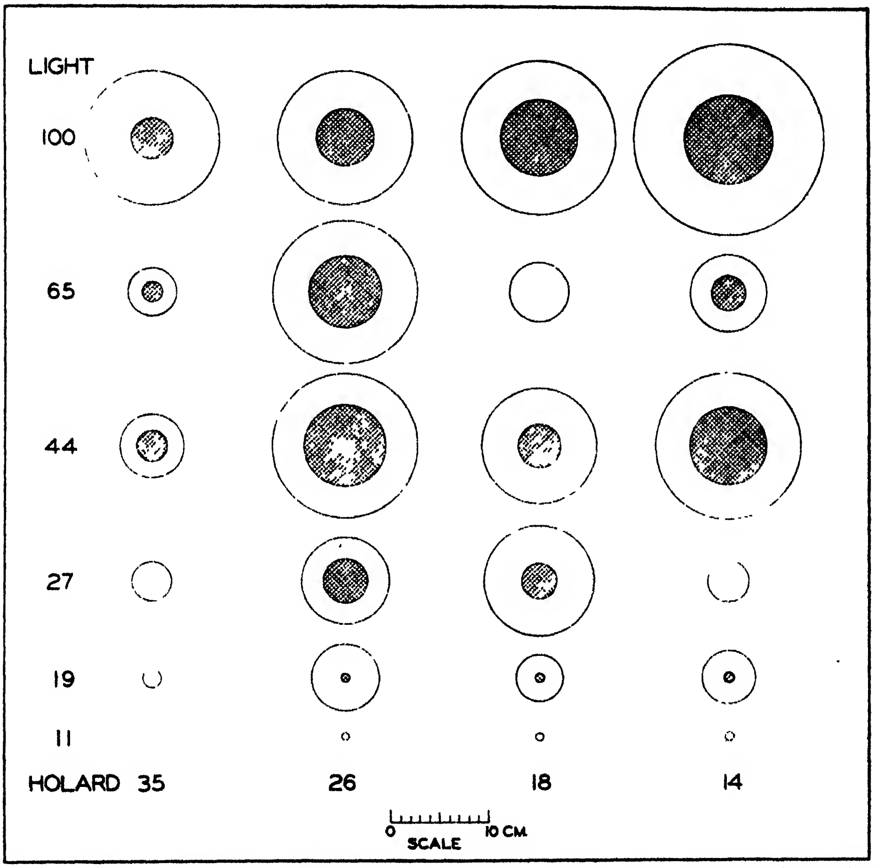

FIG. 3. Comparative diagram of flower heads; disk cross-hatched, rays plain, buds clear.

Wet weight of plant exhibited a complete correspondence with holard, falling off with much uniformity from 35 to 14 per cent., while the dry weight was equally consistent. This was likewise true for the averages, the respective values for the six light intensities from sun being 85.4, 72.2, 63.1, 43.8, 17.5, and 5.3 gm. When the organs of the plant were weighed separately, the wet weight of the stem decreased consistently, as did that of leaves and roots with but one or two slight discrepancies. The dry weights of all three gave similarly accordant results, the maximum of 83.8 gm. for

the stem falling in 100/35 per cent. and the minimum, 1.3 gm., in 11/14 per cent., the averages for the light extremes being 52.5 and 2.7 gm. The maximum for the leaves was 29.1 and the minimum 1.1 gm., with the average for the sun 20.4 gm. and for 11 per cent. light, 2 gm. The highest value for roots fell at 35 per cent. holard in sun, namely, 34.7 gm., while the lowest, 0.3 gm., was found at 11/14 per cent. The average weight was 16.5 gm. for all holards in the sun, dropping to 11.4, 9, 5.8, 1.4, and 0.46 gm. in the descending sequence of light intensities. The roots of plants in sun and 65 per cent. light formed very compact masses, but there was a distinct reduction in the number and length of the branches in 44 per cent. This response was much more marked in 27 per cent.; in 19 per cent. the rootlets were reduced to a tuft at the base of the tap root and in 11 per cent. they had all but disappeared (fig. 1). In all cases but in the sun, the weight of the flower heads was greatest in 18 per cent. holard, with 14 per cent. second; while the averages for the five lath-huts fell from 7.3 gm. for 65 per cent. through 5.4, 0.59, 0.24, to 0.018 for the lowest light value. However, owing to the vicissitudes mentioned earlier, these figures are largely provisional (fig. 3).

The course of growth in stem height and leaf area, in other words the march of elongation and expansion, is shown graphically in figures 4 and 5 for the spring series which ended June 4. Since there were 24 variables involved, only a few of the curves could be shown and hence those for holards of 14 and 26 per cent. and light of 11, 44, and 100 per cent. were selected as representative of the entire series. The curve for stem height shows that little differentiation took place during the first two weeks, but by the end of the third week the plants in 44 per cent. light and 26 per cent. holard were one-fourth taller than the others and held this lead to the close of the experiment. At the two extremes of sun and 11 per cent. light the plants did not begin to diverge until the fifth week, when stems in the sun drew away from those in the shade. Leaves likewise exhibited little differentiation during the first two weeks, but a week later the plants with higher holard and light had acquired about twice the leaf area of the others. Expansion continued at much the same rate until the sixth week, when it became definitely greater for the 44 per cent. light. The lowest holard counterbalanced higher light values and the individuals in sun and 44 per cent. light did not diverge until the ninth week.

The influence of size of container was made the subject of a special inquiry, which took account of free phytometers as well as the four types of can indicated earlier. The standard container when complete weighed 15 kg., the intermediate 12 kg., and the smallest 4.5 kg., while the garbage cans reached a total of 110 kg. when filled. The latter were employed as controls, chiefly for the batteries of free phytometers which could not be

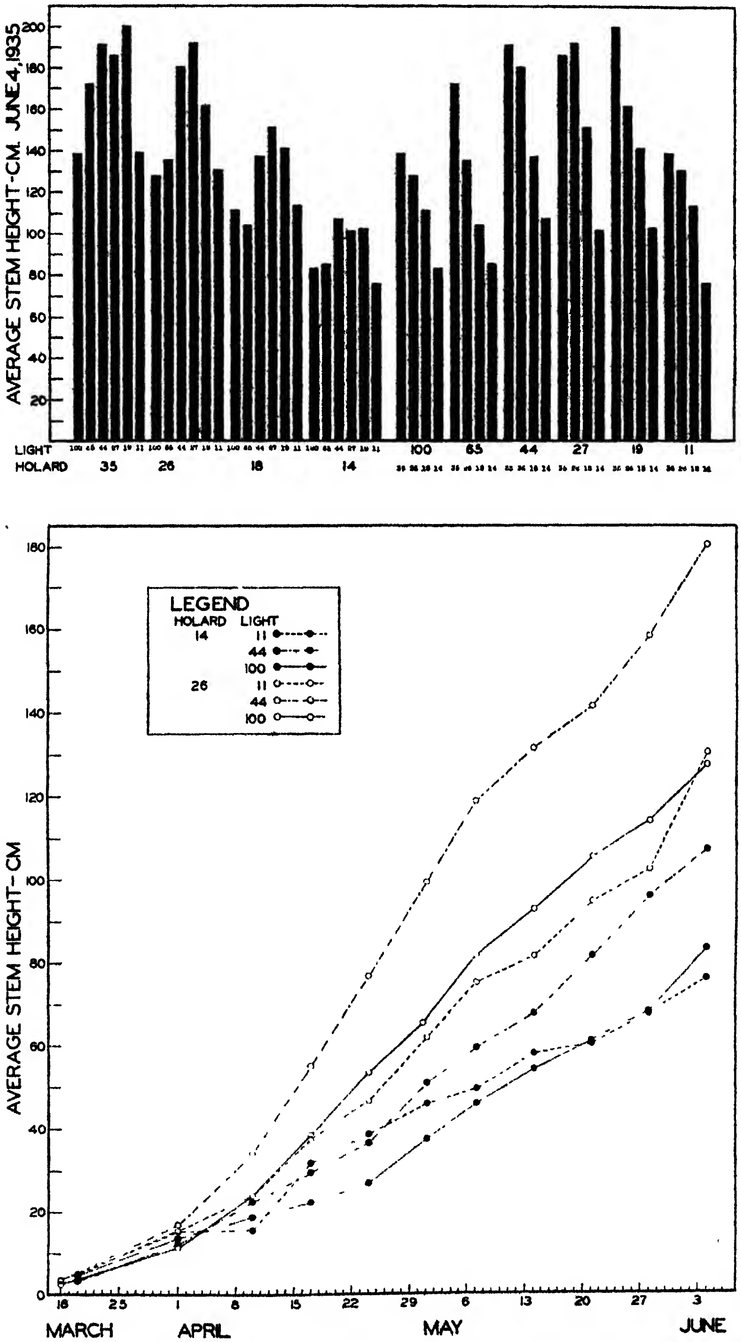

FIG. 4. March of average stem height for series 2, and final values on June 4.

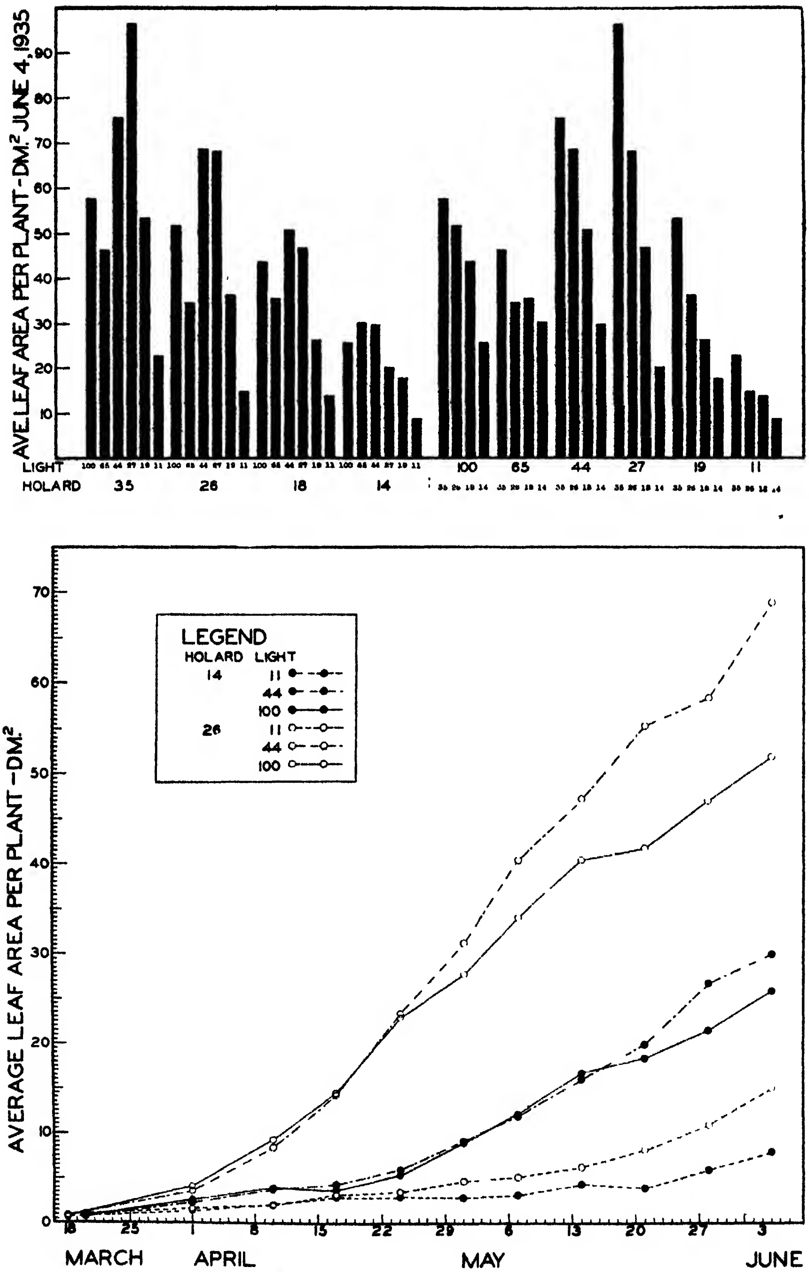

FIG. 5. Increase in leaf area during series 2, and final values on June 4.

weighed, and were but six in number, all placed in the sun. The three sizes of containers amounted to 348 in number, of which 148 belonged to the large phytometer, there being 24 in each light intensity and 6 in each holard. A measured amount of water was given each day, after they had attained a certain size, to the free phytometers in the soil under the six light conditions, and the garbage cans were treated in the same manner. This amounted to giving both sets practically all the water they could use; this fact probably has some bearing upon their slower growth at first when aeration may well have been a factor, as well as upon their much more rapid growth later.

As a consequence, no strict comparison can be made between the growth of these plants and those in the standard containers. They do confirm the experience derived from many phytometer series through two decades to the effect that number and size of container must always be balanced against each other. With 24 sets of conditions, as in the 9 series that have dealt with elongation and expansion, phytometers of 15 kilos. constitute the practicable maximum for a number of 200 or more that will require weighing two to three times daily before the experiment is completed. On the other hand, such a capacity sets a definite limit to growth in the better conditions of light and holard, and phytometers of this size have a dependable life period of but two to three months. By contrast, the garbage-can type of the larger sizes permits carrying such large plants as sunflower and corn through to maturity, but as phytometers to measure a considerable range of conditions, especially in nature, they demand a staff and equipment all but impossible to realize.

A comparison of the growth in the three sizes of phytometer cans at the end of the first three weeks gave a stem height respectively 35 and 40 per cent. greater in the medium and large containers than in the small ones. Likewise, the stem diameter was 21 and 35 per cent. and the number of leaves 40 and 50 per cent. greater, while the leaf area was 133 per cent. more for the medium cans and 52 per cent. more for the large ones. The small phytometers were dismantled at this time as they were evidently reaching the limit of container capacity, and the medium ones were discontinued on May 1 for the same reason. At this time the individuals of the large set were 11 per cent. taller and of the free series 9 per cent. shorter than those in the medium containers and the garbage cans, but in contrast the diameter of the stem was 19 and 14 per cent. more in the large and garbage cans than for the medium and free phytometers. The average number of leaves was 21 for the three sets, but only 14.6 for the free phytometers, in which the leaf area was 24 per cent. less than for the medium cans, while it was 2–4 per cent. greater in the other two. From this time the plants in the garbage cans began to forge ahead and the free phytom-

eters to grow more rapidly than those in the large cans. At the end of the experiment on June 4, the relative values were as follows for the large, free, and garbage-can phytometers: stem height 100, 116, 136; stem diameter 100, 131, 167; number of leaves 100, 128, 149; leaf area 100, 237, 429.

The remaining three sets were taken off to permit determining wet and dry weights at the end of 12 weeks, with the results shown in table III.

TABLE III

WET AND DRY WEIGHTS OF THE THREE FINAL SETS

	PERCENTAGE LIGHT					
	100	65	44	27	19	11
	WET WEIGHT					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Large cans	627.1	507.3	577.6	499.5	295.0	96.3
Free phytometers	1394.9	1265.5	1239.6	323.4	142.6	29.6
Garbage cans	2340.8					
	DRY WEIGHT					
Large cans	127.7	105.4	105.5	61.8	24.9	7.5
Free phytometers	204.8	168.7	127.9	43.7	13.8	2.0
Garbage cans	438.3					

For both wet and dry weights, the values for the garbage-can controls were approximately twice as high as for the free phytometers, and for these about twice as great as for the large phytometers, in the case of sun conditions. Both of the latter fall off consistently in weight with decreasing light intensity, and most rapidly in the lower intensities. The large cans begin to exceed the free plants at 27 per cent. light and both wet and dry weights are more than three times greater in 11 per cent. The cause of this reversal in behavior requires further investigation, as does also the striking difference in the three sets in the sun. This bespeaks better conditions in the larger soil masses, partly to be explained by the generous watering, and the drop in the free phytometers is most plausibly explained by the effect of this upon air content in the three lower light values.

Structural modifications

In addition to the striking responses in growth and form of stem, leaf, and roots, corresponding or compensating adaptations occurred in the intimate structure of these organs. These were especially exhibited by the stomata, as seen in tables IV and V.

The epidermis was stripped from the leaf and immediately placed in absolute alcohol in accordance with the usual technique, a median leaf being selected in each of the 24 sets. The strips were taken from the proximate

TABLE IV

NUMBER OF STOMATA PER SQ. MM. IN UPPER AND LOWER EPIDERMIS THROUGHOUT THE LIGHT AND HOLARD SERIES

Light	Holard 35 per cent.		Holard 26 per cent.		Holard 18 per cent.		Holard 14 per cent.		Average number	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
%										
100	315	288	341	280	258	240	248	240	290.8	262.1
65	293	286	221	221	228	218	203	195	236.2	230.1
44	165	174	180	184	195	195	180	190	180.1	185.9
27	120	129	145	146	142	147	135	140	135.5	140.5
19	105	120	116	125	165	165	131	135	129.4	136.4
11	90	116	90	117	95	112	123	138	99.6	120.8
Ave.	182	186	182	179	181	180	170	173	178.6	179.3

center of the leaf, about halfway from the base to tip and from midrib to margin. The major correlation in number of stomata is with light intensity, the decrease from 100 to 11 per cent. being all but entirely consistent for both lower and upper surfaces in each holard and perfectly so for the averages of each light value (fig. 6). These range from 291 in 100 per cent. to 99 in 11 per cent. for the lower epidermis and 262 and 120 for the upper. The averages for the four holards are not significant except in the 14 per cent., since averaging obscures the most novel point in the behavior as to number. This is the fact that stomata are most numerous in the lower epidermis for 100 and 65 per cent., but tend to become slightly more abundant in the upper for the next two intensities and definitely so in the two

TABLE V

SIZE OF STOMATA IN MICRONS FOR THE LOWER EPIDERMIS OF PLANTS THROUGHOUT THE SERIES

Light	Holard 35 per cent.	Holard 26 per cent.	Holard 18 per cent.	Holard 14 per cent.	Average size
%	μ	μ	μ	μ	μ
100	34.4 × 24.1	20.1 × 24.8	31.8 × 23.7	28.3 × 21.2	28.7 × 23.5
65	24.7 × 24.1	32.9 × 23.4	23.8 × 21.2	28.3 × 21.2	27.4 × 22.5
44	31.8 × 21.2	34.3 × 22.3	28.3 × 21.2	26.6 × 21.2	30.6 × 21.5
27	33.3 × 26.6	33.6 × 21.2	30.4 × 23.4	28.3 × 21.2	31.4 × 23.1
19	33.6 × 24.8	31.8 × 24.8	31.2 × 21.6	30.1 × 21.2	31.7 × 23.1
11	34.0 × 24.4	31.8 × 24.8	29.4 × 23.0	28.3 × 21.2	30.9 × 23.4
Ave.	32.0 × 24.2	31.6 × 23.6	29.1 × 23.0	28.3 × 21.2	30.0 × 22.8

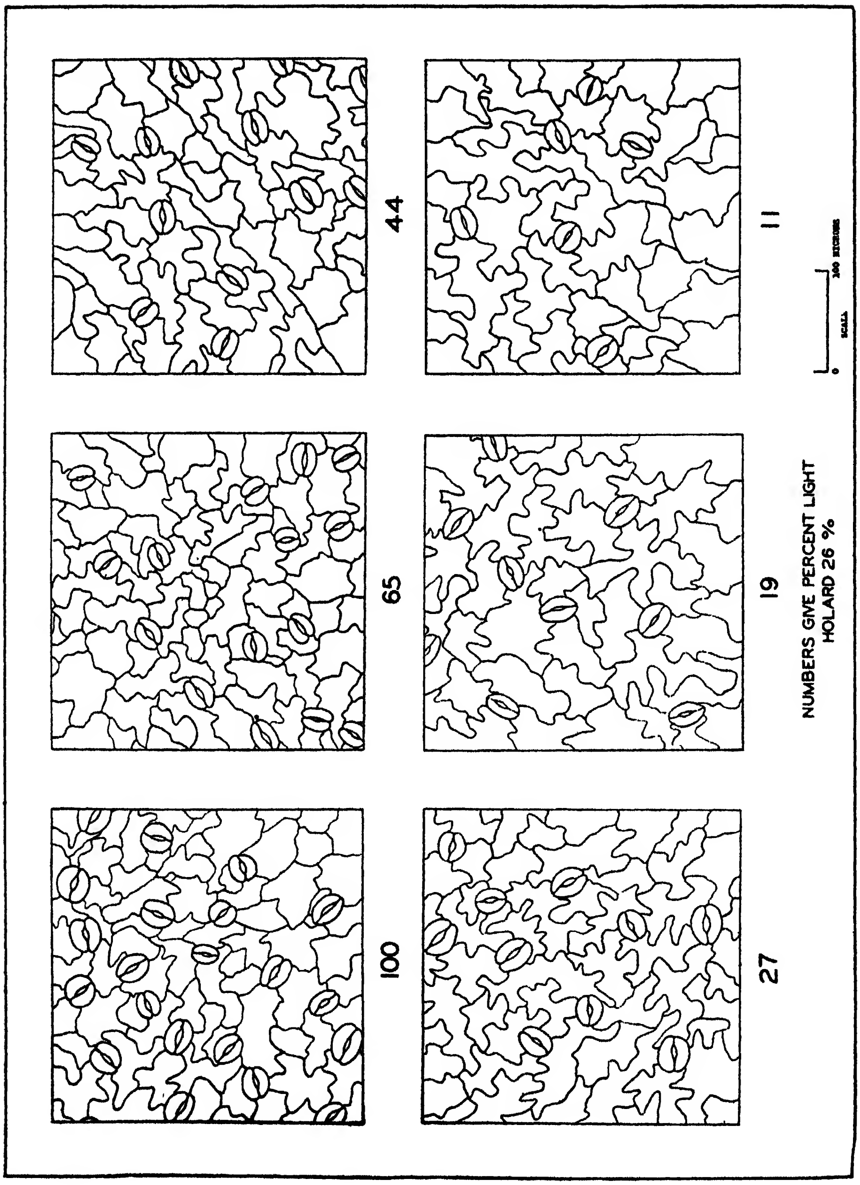

FIG. 6. Modification of stomatal number in the six light intensities.

lower values. While the numbers generally follow the relative expansion of the leaf, the change in preponderance from lower to upper surface makes it clear that the number of stomatal initials varies under the several conditions, not merely between surfaces but also between leaves and to a considerable extent independent of the degree of expansion.

Modification in size bears no evident relation to light intensity in any particular holard, but the average size for the three higher intensities is distinctly less than for the three lower ones. The chief difference, however, is to be found in the averages for the respective holards, the largest size occurring in 35 per cent. and this diminishing regularly to 14 per cent.

Quantitative changes in the mesophyll are reflected in the thickness of leaf, while the qualitative ones naturally affect the mesophyll, either as direct responses to light and water or indirectly in relation to stomata and air passages. As may be seen in table VI, the thickness of the leaf was

TABLE VI

THICKNESS OF LEAF, PALISADE AND SPONGE TISSUES

Factor gradients		Leaf	Palisade	Sponge
Light	Holard			
%	%	μ	μ	μ
Large cans				
100	35	267	153	114
	14	192	111	81
65	35	248	142	106
	14	177	106	71
11	35	142	96	46
	14	142	78	64
Free phytometers				
100	35	238	131	107
65	35	230	126	104
44	35	202	128	74
27	35	156	92	64
19	35	149	78	71
11	35	149	89	60
Garbage cans				
100	35	258	135	124

greatest for the large cans in sun and 35 per cent. holard, followed in order by garbage cans, large can in 65/35 per cent., and free phytometers in sun and 35 per cent. The general agreement between the large and free phytometers was close, the two falling off consistently and in much the same

degree from a maximum in sun to a minimum in 11 per cent. light. For the large plants, 35 per cent. holard produced a leaf one-third thicker than 14 per cent., except for the lowest light intensity, where the values were the same. In this set the values for palisade and for sponge decreased

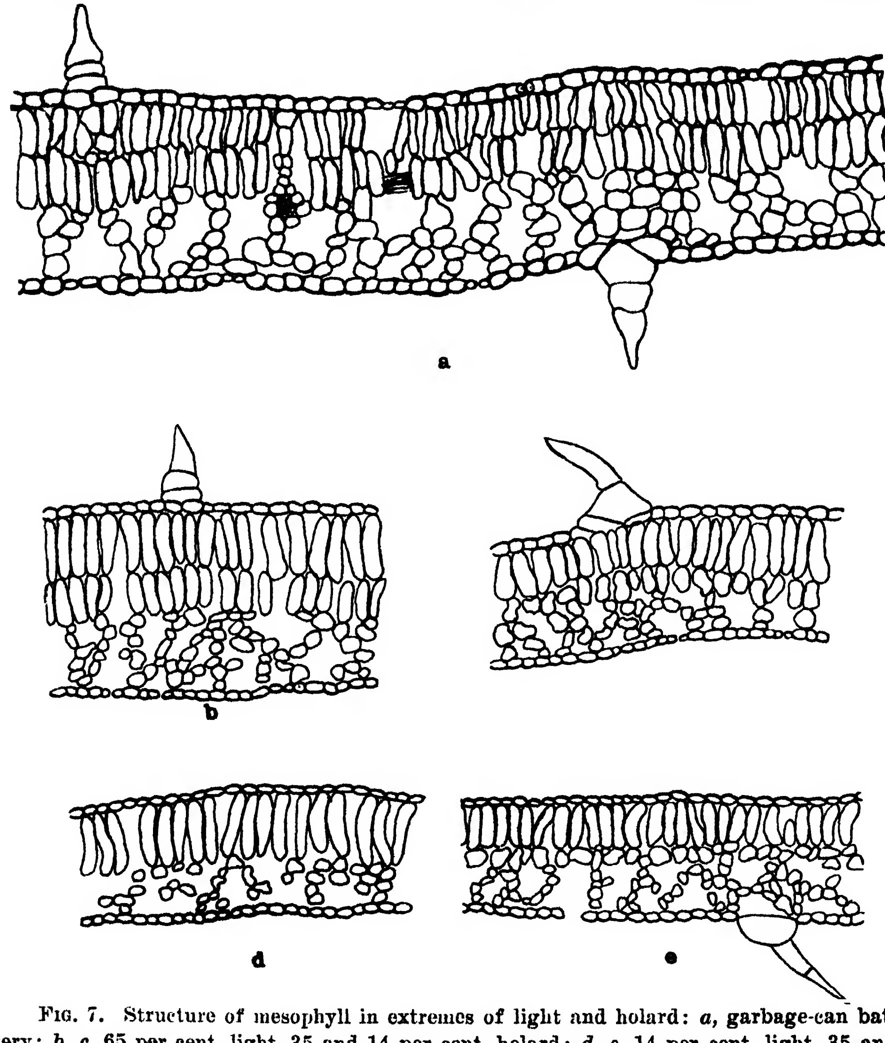

FIG. 7. Structure of mesophyll in extremes of light and holard: *a*, garbage-can battery; *b, c*, 65 per cent. light, 35 and 14 per cent. holard; *d, e*, 14 per cent. light, 35 and 14 per cent. holard. $\times 150$.

regularly with reduced light, except for 11 per cent., and with this same discrepancy, each tissue was thicker in 35 per cent. than in 14 per cent., in accord with the behavior of the leaf itself. The free phytometers were somewhat less harmonious, but there was general reduction from sun to 11 per cent. light.

The mesophyll likewise exhibited structural modifications in close correlation with the factor dosage, as illustrated in figure 7. With the cross section of a median leaf from a garbage-can phytometer as the standard, a large-phytometer leaf from 65/35 per cent. agrees in having two full rows of palisade cells and essentially the same type of sponge tissue. However,

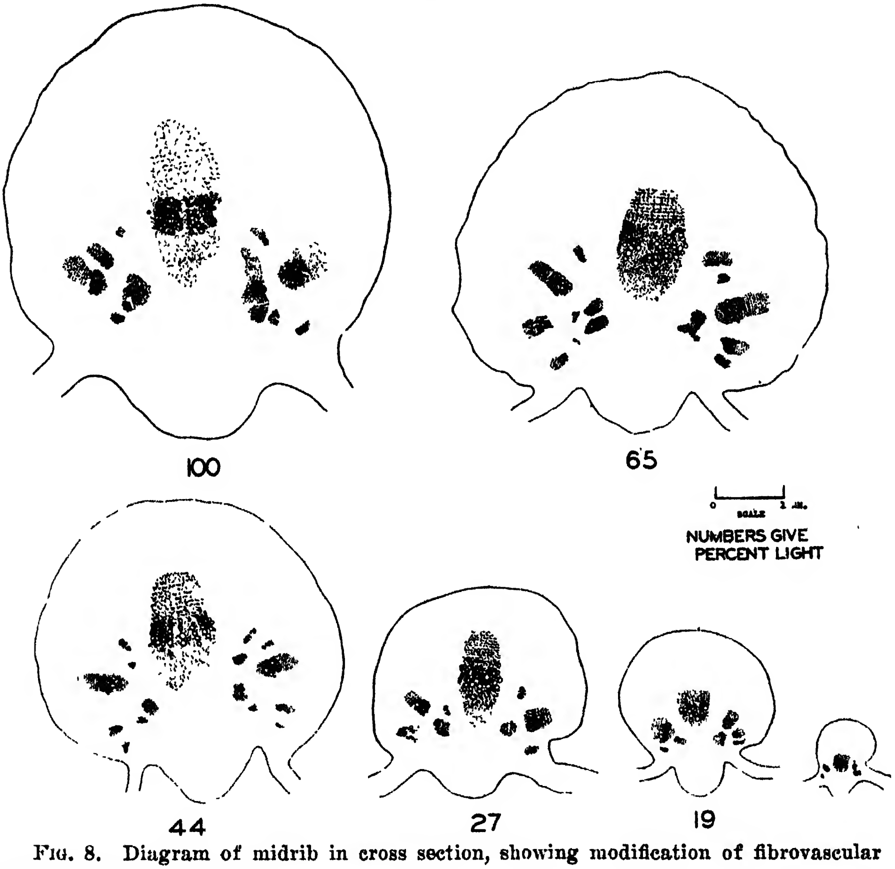

FIG. 8. Diagram of midrib in cross section, showing modification of fibrovascular system; 26 per cent. holard.

leaves in the same light intensity but in 14 per cent. holard had reduced the inner palisade cells to about half the length and the sponge also in some measure. In 11/35 per cent., the inner row of palisade had completely disappeared and the sponge tissue was much looser in texture, while 14 per cent. holard in the same light produceed a shorter outer row of palisade, with the inner reduced to globoid cells.

Cross sections of representative portions of the midrib were employed for comparison of the modification of the conductive-supportive tissues. These differences are best appreciated in the graphic form (fig. 8), but

an effort has been made to express them in quantities also, as shown in table VII.

TABLE VII

MODIFICATION OF THE FIBROVASCULAR SYSTEM IN MIDRIB; 26 PER CENT. HOLARD

Light	Number of strands			Total area in cross section				
	Primary	Secondary	Tertiary	Midrib	Strands	Primary	Secondary	Tertiary
%				sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.
100	1	4	6	0.268	0.036	0.022	0.011	0.0021
65	1	3	11	0.218	0.023	0.014	0.004	0.0055
44	1	2	13	0.147	0.022	0.014	0.003	0.0054
27	1	2	6	0.074	0.011	0.007	0.002	0.0023
19	1	1	6	0.039	0.005	0.002	0.001	0.0012
11	1	0	4	0.008	0.0006	0.0005	0.000	0.0001

The primary bundle of the midrib naturally persists throughout the several conditions, but the secondary strands reflect decreasing light intensity with much accuracy, while the tertiary ones are less consistent, owing in part to the splitting of secondary strands. The adaptation of the fibrovascular system to lessened need for conduction and support with decreasing light intensity is much more evident in the values for cross section areas. For the midrib itself, the area in sun is 30 times greater than in 11 per cent. light, the intermediate figures being entirely consistent. The reduction in the vascular system is twice as great, the total area of the strands being 60 times as much in the sun as in the lowest light intensity, while the sequence of decrease is essentially consistent for total strands, as well as for primary and secondary ones.

The reduction of the number of strands is less consistent for the stem than for the midrib, but the decrease in areas is of the same character, fol-

TABLE VIII

MODIFICATION OF FIBROVASCULAR SYSTEM OF STEM; 26 PER CENT. HOLARD

Light	Number of strands		Total area in cross section	
	Primary	Secondary	Stem	Strands
%			sq. cm.	sq. cm.
100	12	8	0.95	0.198
65	10	6	1.18	0.200
44	10	6	0.88	0.117
27	11	9	0.43	0.062
19	6	6	0.27	0.018
11	8	6	0.1	0.011

lowing the light intensity closely, with the exception of a small discrepancy in 65 per cent. (fig. 9). Moreover, while the stem area at 11 per cent. is seven times smaller than for the sun, that of the strands is 18 times less, or more than twice as much. In general the stem appears to be somewhat more plastic than the midrib, and this assumption is supported by the fact that under the pressure of continuous wind of varying velocities, bundles

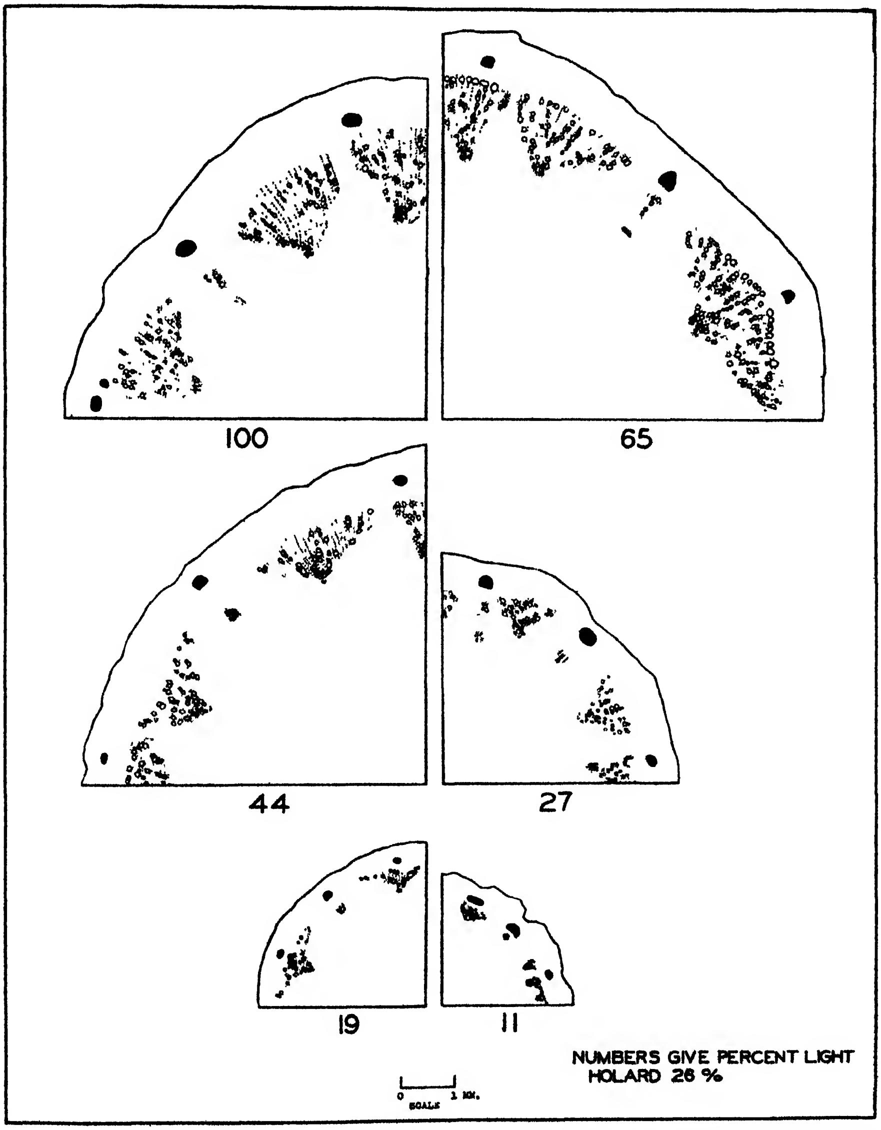

FIG. 9. Diagram of stem quadrants in cross section showing modification of fibro-vascular system.

increase in number relatively more rapidly than in size and the bast strands receive special emphasis.

Summary

1. The conclusions reached heretofore as to the functional and growth responses of *Helianthus annuus* to combinations of light and holard and the relative rôles of the two are fully borne out in two new series with two additional lath-hut intensities, and supported by the behavior of free phytometers in the same light intensities.

2. The much better growth in the garbage-can and the free phytometers is confined to the higher light intensities, and the large phytometers represent the best compromise for sunflower batteries under the conditions that obtained. Comparison of the data appears to leave no doubt that within the range of holard employed, namely, 1 to 2 per cent. above the echard for the soil and up to the point where a tendency toward deficient aeration appears, the phytometers not only utilize water in close correlation with the amount present, but this is also reflected with much accuracy in function and growth.

3. The four plant organs behave essentially as does the entire plant in their response to the 24 different combinations of light and holard.

4. The basic correlation between factor, function, growth as an integrator, and form as the final adaptation, is regularly a close one in the case of *Helianthus annuus.* It appears not only to justify fully the use of standard and free phytometers in measuring in plant terms the varied transplant eces at the Alpine Laboratory and adaptation sequences at Santa Barbara, but also to forecast the responses in function and form of the several hundred species now in process of adaptation.

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OSMOTIC PRESSURE AND WATER CONTENT OF PRAIRIE PLANTS[1]

LAURENCE A. STODDART

(WITH FIVE FIGURES)

Introduction

Many conflicting ideas have been expressed concerning osmotic pressure in plant cells. The purposes of this investigation were to determine the range of osmotic pressure in prairie plants and to ascertain, if possible, any correlation between osmotic pressure and certain ecological factors.

Since the osmotic pressure of a solution is dependent upon the proportion of water and osmotically active substances, it may be concluded that either a decrease in the water content or an increase in osmotically active substances will have an identical effect, namely, an increase in the osmotic pressure.

The plant may be considered as a product of its environment, and the osmotic pressure as an expression of that environment's effect upon the plant. The plant lives in a dual habitat, and acts as a balancing factor between the moister soil surrounding the roots and the drier air surrounding the tops. The plant, as a connecting link between these habitats, expresses their relative forces by its osmotic value because the percentage of water in the plant depends upon the comparative demands of these two habitats. If the soil supplies moisture as fast as the air removes it, then the osmotic pressure must be low. As the air removes more and more water and the water content of the plant tissue is drawn upon in order to supply the demand, then, so long as the osmotically active substances remain constant, the osmotic pressure rises proportionately. Soil moisture and air humidity may therefore be regarded as the most important external factors of the environment, and the water content of the plant tissues may be considered as an extremely important internal factor in the study of osmotic pressure. It is believed that changes in osmotic pressure should be looked upon primarily not as an adaptation to the environment but as a result of environmental changes; thus high pressures in time of drought are chiefly a result of the drought and not an adaptation to it.

During the extremely dry summer of 1934 osmotic variations due to changes of water content far overshadowed those due to changes of sugar content, therefore the latter factor was not considered in any detail.

[1] Contribution from the Department of Botany, University of Nebraska, no. 95.

Literature

One of the most complete summaries of recent investigations on osmotic pressure is that given by MILLER (**17**). KORSTIAN (**11**) and MEYER (**16**) give good accounts of the methods of collecting material. For information on killing of material and extraction of sap, the papers of DIXON and ATKINS (**1**), GORTNER, LAWRENCE, and HARRIS (**4**), and MEYER (**16**) have been found helpful. Osmotic pressure as an ecological or environmental indicator is well treated by DRABBLE and DRABBLE (**2**), McCOOL and MILLAR (**14**), KORSTIAN (**11**), MEYER (**15**), HARRIS, LAWRENCE, and GORTNER (**8**), MOLZ (**18**), and EATON (**3**). The indicator value of osmotic pressures is discussed by HARRIS *et al.* (**7**) and by WALTER (**20**). The daily and seasonal cycle was studied by HERRICK (**10**), MOLZ (**18**), McCOOL and MILLAR (**14**), HENRICI (**9**), and KORSTIAN (**11**). HENRICI worked on the osmotic pressure of grasses (**9**). The relationship of osmotic pressure to drought and frost resistance is reviewed by MAXIMOV (**13**). Water content of tissues was studied by YAPP and MASON (**24**) and by McCOOL and MILLAR (**14**). WALTER (**20**) alone has touched upon the osmotic pressures of prairie plants. He made determinations on a dozen species in the early spring of 1930 and again after a moderate midsummer drought.

Methods

All osmotic pressure determinations were made by the cryoscopic method. Material was gathered in as nearly as possible the same place throughout the season, in order to avoid soil moisture heterogeneity. Collections were made on either the lowland or upland prairie at Belmont, 2 miles north of Lincoln, Nebraska, between the hours of 1 and 3 P.M., unless otherwise noted. The plants were cut at ground level and placed immediately in pyrex glass beakers, corked, and frozen in carbon dioxide ice, where they were left overnight. The juice was extracted from the thawed material the following morning at a pressure of 10,000 pounds. The freezing point of the sap was determined at once by use of an ether evaporation device (fig. 1) much as described by MATHEWS (**12**). The thermometer measured to 0.001°, and, by means of close fitting tubes, a test could be made with only 2 to 3 cc. of sap. Atmospheric pressure was calculated according to the customary formula and tables by HARRIS and GORTNER (**5, 6**).

Water content was determined on samples of about 50 gm. collected in screw-top aluminum cans. The samples were dried at 80° C. for at least 48 hours. The calculations were made on the basis of green weight, to conform with other work on water content of plant tissues.

Soil moisture determinations were made at each collection and were designed to show the trend of available moisture rather than the specific

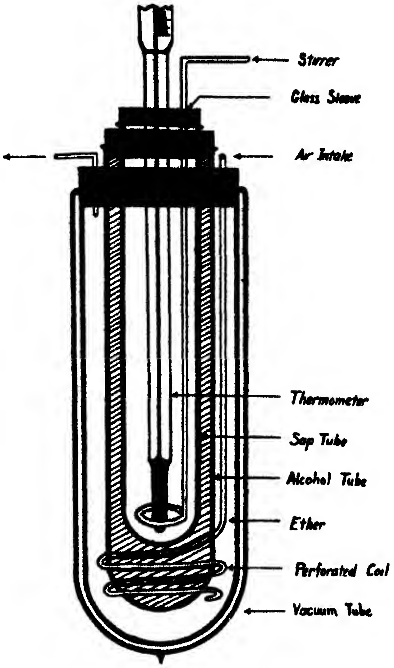

FIG. 1. Ether evaporation freezer used in cryoscopic measurements. Air is sucked from the lefthand tube by means of a water suction pump which causes air to enter at the righthand (intake) tube. The glass stirrer is operated constantly. About 10 minutes is ample time to freeze a sample.

moisture available to any one individual. Samples were taken at depths at 0–6 inches, 6–12 inches, 1–2 feet, and 2–3 feet. The Briggs' geotome and the customary friction-top soil cans were used. Calculations were made on the oven-dry basis.

Procedure

SEASONAL TREND

The summer of 1934 was the hottest and driest on record in Nebraska, and consequently the absolute extremes of values were doubtless met. The seasonal trend of the most important species shows a wide range both in osmotic values and in water content of the tissues. The values for shallowly rooted plants especially followed closely the graph of available water in the soil (fig. 2). The available water decreased throughout most of the season except for a period in early June when slight increases were recorded. The soil moisture in all levels down to 3 feet reached the hygroscopic coefficient, which was taken as the limit of availability. Water in some levels dropped to 2 per cent. below the hygroscopic coefficient.

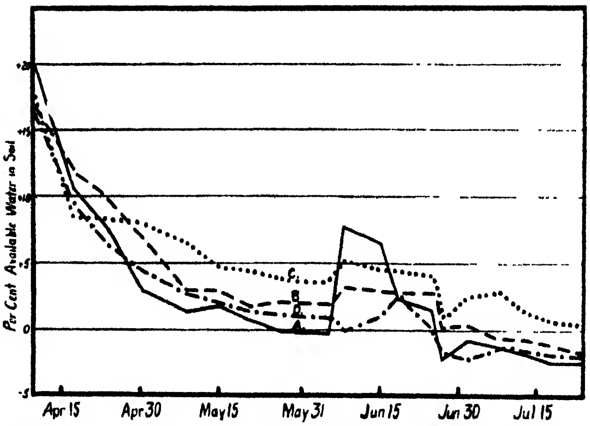

FIG. 2. Available soil moisture in the first 3 feet of upland prairie soil: *A*, at 0 to 6 inches depth; *B*, 6 to 12 inches; *C*, 12 to 24 inches; and *D*, 24 to 36 inches.

Relative humidity was always below 30 per cent. when samples were taken, and readings as low as 12–15 per cent. were encountered in July.

Table I includes a group of plants which succumbed in early summer to the severe drought. All the species blossom and fruit early in the season, and then most of them either dry or remain almost dormant in the shade of the tall grasses. Their osmotic pressures increased as the season progressed and the soil dried. While some of these grasses had ranges of 20 to 38 atmospheres, the forbs had usually a rather low range. Most of the early readings in this group were higher than average. In all cases the water contents varied inversely as the osmotic pressures.

Perhaps the most outstanding feature of the season's data is the fact that *Rosa arkansana, Liatris punctata, Kuhnia glutinosa, Psoralea floribunda,* and others, having roots 10 to 20 feet in depth (**21, 22**), did not show a great increase in osmotic pressure even when drought was most severe (table II). The maximum increase in this group was about 12 atmospheres, although some individuals showed almost no definitely increasing trend. The water content of the tissues decreased regularly although the decrease was less than that shown in more shallowly rooted species. Experiments have shown that prairie soils, although they may dry below the point of available water in the surface layers, are always moist at deeper layers (**23**).

Moderately deeply rooted upland plants, however, such as *Helianthus rigidus, Andropogon scoparius, Bouteloua gracilis, Solidago glaberrima,* and others, which extend from 3 to 9 feet deep, showed great increases in osmotic pressures as the soil dried (tables III and I). Most of the moderately deeply rooted grasses reached osmotic pressures of 35 or more atmospheres, and the xeric *Bouteloua gracilis* reached 60 atmospheres. Many forbs also showed

TABLE I

OSMOTIC PRESSURES AND WATER CONTENTS OF PLANTS THAT SUCCUMBED IN EARLY SUMMER
EACH DRIED AT THE TIME OF LAST DETERMINATION OF ITS OSMOTIC PRESSURE

SPECIES	CRITERION	APR. 10	APR. 17	APR. 24	MAY 1	MAY 8	MAY 15	MAY 21	MAY 28
Allium mutabile	Per cent. water								
	Pressure		10.8	10.9			9.1		
Anemone caroliniana	Per cent. water								
	Pressure	17.3		18.5					
Antennaria campestris	Per cent. water								
	Pressure		9.9	12.4		18.2			
Astragalus crassicarpus	Per cent. water	84.0		82.3	78.5	74.0	72.9	71.0	69.2
	Pressure	12.4	10.9	13.2	13.3	15.4	15.6	17.9	18.7
Bouteloua gracilis	Per cent. water								37.7
	Pressure				21.6	25.9		43.5	59.5
Carex pennsylvanica	Per cent. water			64.6					
	Pressure	12.8	12.8	19.3			17.3		30.2
Cogswellia daucifolia	Per cent. water	79.7		79.5	77.1	77.3		70.1	63.5
	Pressure	14.4	11.5	14.6	16.1	16.1	16.6	17.0	20.2
Fragaria virginiana	Per cent. water								
	Pressure		12.0					14.9	
Koeleria cristata	Per cent. water								
	Pressure	15.8	16.4	17.4		24.1			35.0
Nothocalais cuspidata	Per cent. water								
	Pressure	12.8	13.2				15.2		
Poa pratensis	Per cent. water		72.5			57.4			
	Pressure	14.5				28.9	25.5		28.5
Sisyrinchium campestre	Per cent. water								
	Pressure				14.4				

increases of as much as 25 atmospheres. The range of osmotic pressures from early spring to midsummer was much greater in every species than that recorded by WALTER (20).

In this group also the water content of tissues varied inversely with the osmotic pressure and very great decreases were common. Many plants when growth ceased had only half as high a water content as had been recorded earlier.

It is evident from tables I–IV that the osmotic pressure and water content may be more or less characteristic of a species. Thus the values for an individual species may vary definitely from those of another in the same habitat. If one species has a higher osmotic pressure than another, in gen-

TABLE II

Osmotic pressures and water contents of deeply rooted upland plants. None showed abnormal drying

Species	Criterion	Apr. 19	Apr. 24	May 8	May 21	May 28	June 8	June 20	July 2	July 9	July 18	July 25	July 31
Amorpha canescens	Per cent. water			69.6	62.5	59.3	54.2	53.2	50.1	45.8	43.6	42.0	44.8
	Pressure		11.6	13.1	15.8	20.8	16.2	15.1	17.2	20.0	24.4	27.7	22.1
Baptisia leucophaea	Per cent. water				70.6	64.0	64.5	62.3	52.6				
	Pressure	8.6	10.8	12.0	11.4	14.8	16.0	16.7	17.3		24.0	20.6	
Kuhnia glutinosa	Per cent. water								71.2		69.0	63.5	68.3
	Pressure							11.5	12.1		12.4	15.0	11.5
Liatris punctata	Per cent. water						68.5	65.5	64.4	61.2	59.2	57.8	55.8
	Pressure					12.8	13.6	11.4	12.8	12.6	14.0	12.7	13.2
Psoralea floribunda	Per cent. water				71.5	71.1	67.2	63.5	61.7	59.2	55.1	56.2	
	Pressure		12.2	14.2	16.9	17.8	16.0	16.8	15.2	17.2	17.3	17.5	15.8
Rosa arkansana	Per cent. water						54.8	54.5		53.5	51.4		50.3
	Pressure		16.9		19.2		15.5	13.3		20.4	20.4		14.8

TABLE III

OSMOTIC PRESSURES AND WATER CONTENTS OF MODERATELY DEEPLY ROOTED UPLAND PLANTS, EACH OF WHICH DRIED AT TIME OF LAST DETERMINATION SHOWN

SPECIES	CRITERION	APR. 10	APR. 19	APR. 24	MAY 1	MAY 8	MAY 15	MAY 21	MAY 28	JUNE 1	JUNE 8	JUNE 15	JUNE 20	JUNE 25	JULY 2	JULY 9	JULY 18
Agropyron smithii	Per cent. water							51.5	49.2								
	Pressure	20.0			23.0		30.2	30.5	33.8		34.3		21.4			40.5	
Andropogon furcatus	Per cent. water							71.4			61.0		60.3			42.8	37.3
	Pressure			9.5		11.5	12.2	11.0	13.9		14.2	12.2	16.0			37.7	36.2
Andropogon nutans	Per cent. water					73.2		66.0		54.5	58.3	59.9	55.3	48.2		38.2	35.8
	Pressure			10.9		10.9	11.4	13.6	18.5	19.7	16.4	10.6	16.9	27.7		35.0	
Andropogon scoparius	Per cent. water			77.0	71.2	66.8		62.5	52.9	48.3	57.0	59.3	52.9	48.2	41.5	36.8	36.7
	Pressure		8.1	9.6	9.8	9.9	12.4	13.6	25.3	37.4	18.1	11.5	17.4	23.5	31.3	55.1	
Bouteloua curtipendula	Per cent. water																
	Pressure													26.0		40.4	
Helianthus rigidus	Per cent. water		82.0			78.7				66.3	71.4		64.5	53.4	48.0		
	Pressure	13.2				12.6		13.3	19.9	32.8	15.2		22.9	29.4	36.9		
Liatris scariosa	Per cent. water												60.9		42.2		
	Pressure										20.4		19.8		36.7		
Panicum scribnerianum	Per cent. water																
	Pressure			12.6							16.7		16.8				
Solidago glaberrima	Per cent. water							69.4		64.8	65.3			59.0		52.8	44.2
	Pressure	16.3		12.0			15.2	13.7	12.8	17.8	14.3	12.4	16.8	22.0		28.1	
Sporobolus heterolepis	Per cent. water			66.7				55.8	47.6		51.7	51.8	48.6	46.8	41.8	39.6	37.3
	Pressure		7.4	12.4	13.7		16.3	18.7	23.4		22.0	15.2	21.5	30.6	37.5		
Stipa spartea	Per cent. water				63.0		62.1	55.7	53.6		47.2					38.8	
	Pressure	15.6	18.2		16.9		16.4	17.3	18.4	24.9	21.8	20.0		25.5		32.7	

eral it can also be expected to have a lower water content. A high or low osmotic pressure may be characteristic of a species, but by extreme variation of available soil moisture the ranges of any two plants may be seen to overlap widely.

It is interesting to note that the prevernal and typically first layer plants, such as *Anemone caroliniana, Carex pennsylvanica,* and *Antennaria campestris* (table I), did not have the markedly lower osmotic pressures that were expected from their characteristically mesic habitat. This, however, may have been due in part to the unusually dry year.

The relationship between water content of the tissue and osmotic pressure should also be noted. In most cases shown in tables I-IV the water content decreased regularly as the season progressed, and, while the water content may have increased during rainy periods, it never responded as much proportionately as did the osmotic pressure. As the plants matured some cells may have dried completely, and having no sap, they would not influence the osmotic pressure, but having dry matter they would tend to decrease the percentage of water in the tissue. There are probably some physiological changes also, such as thickening of the cell walls, which are characteristic of older plants, that may in part account for this fact. As the season

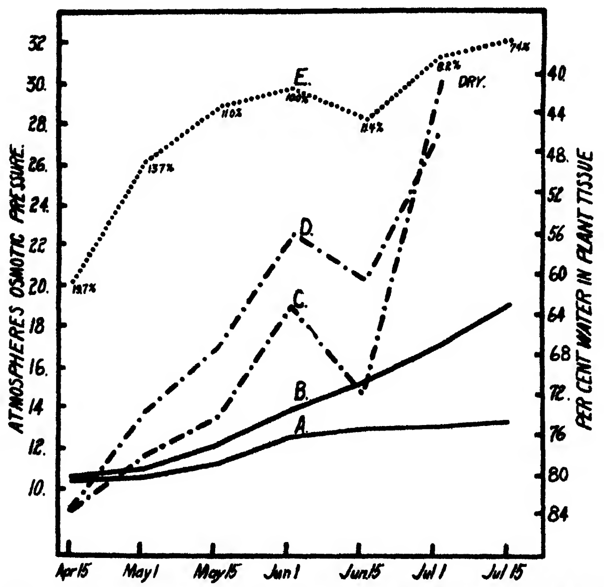

FIG. 3. Graphs of average osmotic pressure (*A*) of *Aster salicifolius, Helianthus grosseserratus, Panicum virgatum, Solidago altissima,* and *Spartina michauxiana,* all of lowland, and water content of their tissues (*B*). Water content (*C*) of *Andropogon scoparius, Baptisia leucophaea, Helianthus rigidus, Solidago glaberrima,* and *Sporobolus heterolepis,* all of upland, and their osmotic pressure (*D*). Average total water content (*E*) of first 3 feet of upland soil.

progressed, therefore, the curve of dry matter tended to deviate steadily from the curve of osmotic pressure. This fact is well shown in lowland plants where little variation in osmotic pressure occurred (graphs A and B, fig. 3).

The small seasonal change in osmotic pressure and water contents of plants listed in table II indicates that their long roots enable them to obtain a sufficient water supply at all times, and that they are the truly drought resistant plants of the prairie. The same tendency is shown in typical lowland plants such as *Helianthus grosseserratus, Aster salicifolius, Solidago altissima, Panicum virgatum,* and others (table IV), for these plants are able to obtain sufficient water because of its greater abundance in the lowland soil. The water table in the lowland seldom falls below 4 feet and water is probably always available below the first 6 inches. Many of these lowland plants show no tendency to increase their osmotic pressure more than one or two atmospheres.

This lack of any very great increase of osmotic pressure in plants of the lowland is further illustrated in figure 3, which shows comparative seasonal curves of five typical lowland and five typical upland plants. The upland plants showed a great increase in pressure as the season progressed while the lowland plants showed a comparatively small increase. The two grasses in each group showed greater increases than did the forbs. That lack of water caused the increase in the upland plants is further borne out in this graph by the fact that their pressures decreased abruptly after the rains of early June. That lowland plants do not have an osmotic pressure typically lower than upland plants, except during times of stress on the upland, is shown by the readings on April 15 at which time the upland plants had the lower average pressure owing to the plentiful soil moisture. Osmotic pressures as an index to site or habitat should therefore be used only during drier seasons.

Responses of deeply and shallowly rooted plants

The same lack of response of plants having access to water is shown by samples of the same species on the same day from both upland and lowland habitats. Figure 4 shows that, in both lowland and upland habitats, the deepest rooted species such as *Rosa arkansana, Liatris punctata,* and *Kuhnia glutinosa,* which penetrate to a depth of 16 to 21 feet, vary but little and sometimes insignificantly both in osmotic value and in water content. In the same two habitats, however, plants such as *Andropogon furcatus, Helianthus rigidus,* and *Solidago glaberrima,* whose maximum root penetration varies only from 7 to 9 feet, show a great difference in both water content of tissues and osmotic pressure. Experiments have shown intermediate values for these plants on midslopes. This shows that with shallower rooted species the dryness of the habitat is indicated by the osmotic pressure and the water

TABLE IV

OSMOTIC PRESSURES AND WATER CONTENTS OF IMPORTANT LOWLAND SPECIES WHICH NORMALLY HAVE AVAILABLE WATER AT ALL TIMES. MOST SPECIES SHOWED NO EFFECTS OF THE DROUGHT

SPECIES	CRITERION	APR. 10	APR. 17	MAY 1	MAY 15	MAY 24	JUNE 1	JUNE 13	JUNE 25	JUL. 2	JUL. 9	JUL. 25	JUL. 31
Aster salicifolius	Per cent. water	83.5	84.2	82.4	80.3	77.6				69.9			65.2
	Pressure	13.0	11.3	9.1	11.4	12.5		12.0	12.7	11.8	11.8	11.4	12.8
Carex vulpinoidea	Per cent. water		69.0	69.0		60.9						33.2	
	Pressure	12.7	13.9	14.5		18.8			19.9			41.1	Dry
Glycyrrhiza lepidota	Per cent. water								65.8			60.0	60.0
	Pressure				13.8		14.5	16.2	16.0			18.8	16.1
Helianthus grosseserratus	Per cent. water			83.6	81.5	80.6		77.0	74.8	76.0	72.0	70.7	71.0
	Pressure		9.2	10.8	11.8	13.2		12.8	12.2	12.6	12.5	11.6	11.5
Panicum virgatum	Per cent. water								65.0		57.5	57.4	56.2
	Pressure			10.1	12.8	10.6	10.6	11.3	10.7		12.0	13.7	12.8
Phalaris arundinacea	Per cent. water				74.4	74.2	72.1	69.9	64.4			59.9	
	Pressure	13.8	14.4	19.2	16.3	16.3	17.4	18.2	17.3			21.5	
Rhus glabra	Per cent. water									78.0			
	Pressure		11.2							10.9			
Solidago altissima	Per cent. water	84.8	82.3	81.1	81.3	74.1		70.6	71.8	67.4	66.8	64.2	64.4
	Pressure	10.3	9.0	10.7	10.2	11.4		12.4	11.3	10.9	10.8	13.2	12.1
Spartina michauxiana	Per cent. water		76.3	72.5	66.3	63.4	58.7	61.2	60.0	55.4	54.7	53.2	
	Pressure	15.7	12.6	12.7	13.2	11.3	16.8	17.9	15.0	19.8	19.7	22.1	
Typha latifolia	Per cent. water		87.0		87.8			79.7	76.7			71.8	
	Pressure		6.2		8.3			10.8	11.8			18.7	

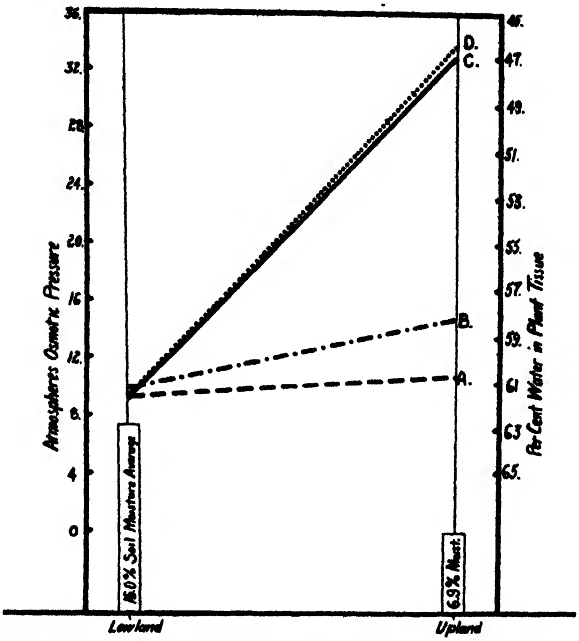

FIG. 4. Average osmotic values of the same species growing on both upland and lowland, from samples collected at the same time. Average osmotic pressure (*A*) of the deeply rooted *Liatris punctata, Kuhnia glutinosa,* and *Rosa arkansana,* and their average water content (*B*). Average osmotic pressure (*C*) of the moderately rooted *Andropogon furcatus, Helianthus rigidus,* and *Solidago glaberrima,* and their average water content (*D*).

content of the tissue. It further shows that in all cases the water available to the plant is indicated both by the osmotic pressure and the water content of the tissue, whether this availability be due to depth and efficiency of the root system or to an abundance of water.

These results may serve to explain the many conflicting reports as to the value of osmotic pressure as a criterion of site. They show that deeply rooted plants should not be used as indicators and that only during times of stress are differences in site expressed. When judiciously employed, however, osmotic pressure is an excellent index.

HUMIDITY AND SOIL MOISTURE AS FACTORS INFLUENCING OSMOTIC PRESSURE

An experiment was conducted to determine the relative influence of humidity and soil moisture. Four closely adjacent plots with similar vegetation, each about 2 meters square, were selected. Two were watered by means of sprinkling during a period of three days. This wet the soil to a depth of 3 feet and increased the average soil moisture from 11.9 to 19.1 per

cent. One of these watered plots and one of the unwatered ones were walled in with stiff cardboard to a height of about 3 feet, and the tops covered with muslin. The muslin transmitted enough light for photosynthesis but afforded sufficient shade and protection from wind to raise the humidity considerably. Inside the enclosures were pans of water which had saturated muslin strips running from them to the muslin roof. This device was sufficient to raise the relative humidity from an average of 33 per cent. outside to an average of 51 per cent. inside the wet plot and 46 per cent. inside the dry plot. These humidities, or at least similar humidities, were maintained for at least 48 hours before samples were taken. No free water was present on the plants when collected (table V).

TABLE V

AVERAGE OSMOTIC PRESSURES FROM TWO EXPERIMENTS DESIGNED TO SHOW RELATIONSHIP OF OSMOTIC VALUES TO HUMIDITY AND SOIL MOISTURE

SPECIES	DRY, NON-HUMID	WET, NON-HUMID	DRY, HUMID	WET, HUMID
	atm.	*atm.*	*atm.*	*atm.*
Stipa spartea	24.9	20.6	18.9	19.2
Sporobolus heterolepis	15.3	14.8	12.0	10.7
Amorpha canescens	16.5	16.3	13.5	13.2
Solidago glaberrima	15.1	14.6	13.7	12.8
Andropogon nutans	13.7	13.6	12.6	12.1
Andropogon scoparius	15.7	11.5	12.6	9.8
Average	16.21	14.75	13.57	12.57

It may be seen from table V that both increased humidity and increased soil moisture decreased the osmotic values. When both were increased the effect was considerably greater than when only one was augmented.

It is of interest that the osmotic pressures of the deeply rooted *Amorpha canescens*, which absorbs very little in the surface 2 feet, were scarcely affected by watering the soil. As would be expected, however, they were decreased by increasing the humidity.

In all but one species, *Andropogon scoparius*, the increase of humidity alone gave a greater change than did increase of water content of the soil alone. This fact does not necessarily indicate, however, that soil moisture is a less important factor in influencing osmotic pressure than is humidity. This soil moisture was necessarily mostly near the surface, and at best the indi-

cation would be only that this particular humidity change was more important than the soil moisture change. It does, nevertheless, show the importance of humidity as a factor affecting osmotic value. The osmotic value decreases as the plant tissue increases its water, whether this increase is due to greater absorption or to decreased transpiration. This relationship to water content of tissues is shown in table VI, which gives data for those plants which were sufficiently abundant to yield a water content sample.

TABLE VI

WATER CONTENT OF PLANTS FROM AREAS ON WHICH HUMIDITY AND WATER CONTENT OF SOIL WERE VARIED

SPECIES	DRY, NON-HUMID	WET, NON-HUMID	DRY, HUMID	WET, HUMID
	%	%	%	%
Sporobolus heterolepis ..	51.8	53.5	. ..	56.0
Amorpha canescens . .	52.3	53.2	54.8	54.3
Solidago glaberrima ..	64.8	. ...	65.6	70.7
Andropogon nutans	55.2	55.2	.	60.5
Andropogon scoparius .	54.9	62.1	61.0	63.6

These water content values show the same sequence as do the osmotic pressures, and strongly indicate that the osmotic change was due solely to change in water content and not to any change in photosynthetic or other physiological processes. This probably was due to the extremely hot dry weather, which prohibited stomatal opening. It is concluded, therefore, that both humidity and soil moisture are important factors, and that both act through the medium of the water content of the tissues.

DAILY CYCLE OF OSMOTIC PRESSURE AND WATER CONTENT

The daily trend of osmotic pressure in *Andropogon scoparius,* the dominant upland prairie grass, was determined. At the time of the investigation the soil was very dry and the humidity correspondingly low. All grass leaves were folded and did not open even during the night. It seems probable that the stomata did not open during the experiment and that no carbon dioxide was absorbed for photosynthesis. We may conclude, therefore, that the cyclic change was due largely to increase and decrease of the water stress. In order to eliminate the factor of soil heterogeneity, twelve plots were staked off and each of the twelve samples was composed of equal parts from each of the plots. Samples for both water content and osmotic

pressure were taken in duplicate every 2 hours for a period of 24 hours. Figure 5 shows the graphs for these two values together with the temperature and humidity. During this period the osmotic pressures were rising rapidly,

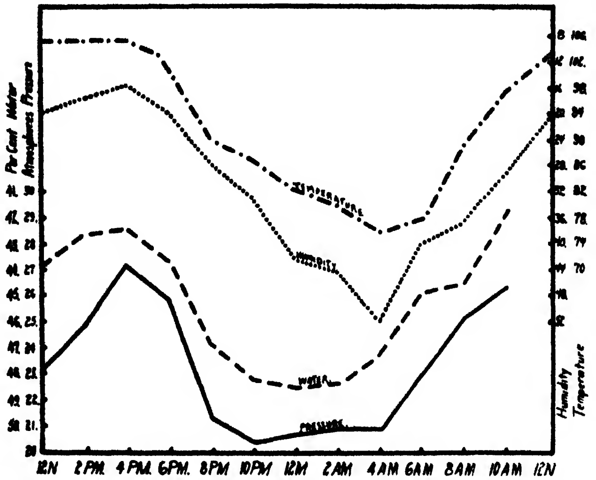

FIG. 5. Daily cycle of water content and osmotic pressure of *Andropogon scoparius* during extreme drought. Relative humidity and air temperature are also given.

and it is probable that on the second day they rose considerably above those on the first day. The graph is much as would be expected, except for the tendency of the osmotic pressure to reach a minimum early in the night and to maintain that minimum until early morning. This may indicate that the osmotic pressure of the plant had decreased to the same value as that of the soil water, and that, in such a case, the value for the plant sap could decrease no further despite the fact that the humidity had ceased to make so great a demand upon the water of the plant. The decrease in the water content of the plant tissues from 2 to 4 A.M. is not clear.

These graphs present further evidence of the importance of humidity as a factor affecting osmotic pressure. They show definitely that a daily curve may be effected by moisture relations as well as by photosynthesis. Since soil moisture varied but little during the experiment and there was probably no photosynthesis, the changes may be attributed to variations in humidity.

A similar daily cycle test was made with the lowland sunflower, *Helianthus grosseserratus*, but in this case, where soil moisture was plentiful, the daily change did not exceed two atmospheres' pressure. Here also the maximum pressure was obtained during the hot afternoon and the minimum during the more humid night. The data show that where soil moisture was sufficient to supply the demands of the atmosphere the daily range was much less than that found in the drier uplands.

Comparative values of roots and tops

Experiments were conducted with *Panicum virgatum* in order to ascertain the gradients of osmotic pressure and water content between the tops and roots. Rhizomes were planted in soil consisting of nine parts of sand and one part of loam. They were allowed to grow until the time of flowering, when the large wooden containers were opened and the roots of one plant were quickly shaken free of adhering sand and frozen for osmotic pressure determination. The osmotic pressure of the top of the plant was also determined. A second plant was used for ascertaining the water content of both roots and top. So far as possible the roots were shaken free from sand before drying. Water content of the sand-soil mixture was also determined so that calculations of the water held by the sand adhering to the roots might be made. When the sample of roots was dry, it was reweighed to get total water loss. It was then washed free from all sand and soil. The weight of the roots and the weight of this adhering sand and soil were next determined. The part of water that had been lost by the adhering soil and the part lost by the roots were then obtained. Finally the water content of the roots was calculated. The water contents of the roots and top were 74.5 and 59.9 per cent. respectively, and the osmotic pressures 8.43 and 13.84 A. Thus the osmotic pressure was greater in the top of the plant, and the water content varied inversely as the pressure.

Effect of age of tissue on osmotic pressure and water content

Late in the season, when most plants had reached a very high osmotic pressure, a plot was watered artificially for a period of three weeks. Some of the area was clipped prior to watering to allow new vegetation to be produced while other parts were left undisturbed in order to preserve the old vegetation. Four species gave enough new growth to permit a test in which the tissue was two weeks or less of age. A determination was also made on old growth in which only mature tissues were used. The results are shown in table VII. It may be seen that in all cases the older tissue had a higher osmotic pressure, despite the fact that the soil moisture was identical in each case. In the two plants tested the water contents varied inversely as the osmotic pressures, as would be expected. This experiment indicates that osmotic pressure increased as the tissue aged regardless of water content of the soil, although in some species, such as the grasses, the increase was very small.

It is of interest to note that on the watered plot the old tissues in many plants decreased their osmotic pressures to values such as were recorded in early spring, and the water content in the plant tissue rose to almost as high a figure as had been recorded early in the season. The average soil moisture

TABLE VII

OSMOTIC PRESSURE OF NEW AND OLD TISSUES UNDER SAME SOIL MOISTURE CONDITIONS

Species	New tissue		Old tissue	
	Osmotic pressure	Water content	Osmotic pressure	Water content
	atm.	%	*atm.*	%
Amorpha canescens	12.88	..	19.60	43.5
Andropogon scoparius	9.39	71.3	9.75	68.5
Solidago glaberrima	10.48	75.7	16.72	58.2
Sporobolus heterolepis	12.76	.	13.60	62.3

in the first 5 feet was only 10 per cent., about 3 per cent. above the hygroscopic coefficient, although the surface soil contained 19 per cent. The generally high humidity at this time likewise was conducive to low osmotic pressures. The deeper rooted species showed much less response than did the shallower rooted ones. Table VIII shows a few of these values with previous high and low readings for comparison.

It is evident from table VIII that the osmotic pressure can be reduced to the spring value, or very nearly so, with an increase in soil moisture. Old tissue tends to be slightly higher in osmotic value than new tissue. As has already been pointed out, however, the water content of the tissue can never be brought back to the high value of spring because of permanent physiological changes and possibly death of some cells.

TABLE VIII

OSMOTIC PRESSURES AND WATER CONTENT VALUES ATTAINED ON A WATERED PLOT, WITH PREVIOUS HIGH AND LOW READINGS

Species	Watered plot		Late summer		Early spring	
	Osmotic pressure	Water content	Osmotic pressure	Water content	Osmotic pressure	Water content
	atm.	%	*atm.*	%	*atm.*	%
Amorpha canescens	19.60	43.5	27.75	42.0	11.68	69.6
Andropogon furcatus	10.36	.	35.04	37.6	10.96	77.0
Andropogon nutans	9.99	71.4	37.76	35.8	9.51	73.2
Andropogon scoparius	9.75	68.5	37.47	36.7	8.19	77.0
Bouteloua gracilis	12.88	.....	59.50	37.7	....	...
Helianthus rigidus	11.28	....	36.95	48.0	9.51	82.0
Liatris punctata	11.80	59.8	14.08	57.8	11.44	68.5
Solidago glaberrima	16.72	58.2	28.46	44.2	11.04	75.2
Sporobolus heterolepis	13.60	62.3	37.54	37.3	7.47	66.7
Stipa spartea	16.72	.. ...	32.77	38.8	15.64	63.0

Discussion

Osmotic pressures and water content of tissues closely parallel each other, and both are highly indicative of the availability of water. The fact that one species may develop a high osmotic pressure while that of another remains low implies that the first is suffering from water shortage while the second is not. Thus the high pressure is deemed to be a result of drought and not an adaptation to it. The plant's ability to increase its osmotic pressure and thus keep the osmotic gradient in its favor is doubtless a valuable feature during periods of stress. It is rendered less valuable, however, by the fact that any possible increase will enable the plant to absorb water from a soil but little drier than that from which it could extract water without increase in pressure. This is due to the fact that the osmotic pressure of the soil solution, which is normally below that of even the most mesic plants, as it approaches the point of non-availability rises very rapidly to extremely high values not attainable by plants (**19**, p. 25). It is also possible that slowness of water movement may cause wilting even while the osmotic pressure of the plant is still greater than that of the soil solution (**19**). In this case osmotic pressure of the sap would never be a factor involved in drought resistance.

Colloidal imbibition is capable of exerting much greater force than is osmotic pressure and is probably the more important factor acting to secure water from a dry soil (**19**).

These studies emphasize the fact that the drought resistance of prairie plants is due largely to their extremely deep and efficient root systems.

Summary

1. Osmotic pressure is regarded as an expression of the environment, indicating the relative forces with which the soil supplies water to the plant and the air removes it through transpiration.

2. As the soil dried with the progress of the season the osmotic pressure of all prairie plants studied increased. This increase was frequently 30 or more atmospheres among moderately deeply rooted upland plants. Among deeply rooted upland plants and plants growing in low moist habitats the increase was usually only 2 to 10 atmospheres.

3. As the soil dried, the water content of the tissue very regularly decreased, although the change was much less marked in lowland plants and in those having very deep roots.

4. Water content of plant tissue is a rather exact indicator of osmotic pressure, plants with high pressures usually having low water content and plants with low pressures usually having high water content.

5. Osmotic pressure of the sap and water content of the tissue responded

readily to increases in soil moisture, but the response was much less in the case of water content of tissue.

6. Osmotic pressure and water content of the tissue, except in very deeply rooted plants, are both excellent criteria of the water content of the habitat.

7. Variation of both humidity and soil moisture indicated that these factors had a great influence on both osmotic pressure and water content of tissues. Minimum osmotic pressure and maximum water content of tissues were attained with a high humidity and a high soil moisture.

8. Determinations of both osmotic pressure and water content of *Andropogon scoparius* every two hours during a day and a night showed a reverse correlation between osmotic pressure and humidity, and a direct correlation between humidity and water content of the tissues.

9. Similar determinations on a lowland species, *Helianthus grosseserratus,* rooted in moist soil, showed much less daily variation.

10. Roots of *Panicum virgatum* had a lower osmotic pressure and a higher water content than the tops.

11. New growth of plant tissue had a lower osmotic pressure and a higher water content than old growth.

12. Artificial watering in late summer lowered osmotic pressure and increased water content of tissues. The values approached those of spring.

13. Soil moisture and humidity are considered the major factors affecting osmotic pressure.

The writer wishes to express sincere appreciation to Dr. J. E. Weaver, under whose direction this work was done.

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KINETICS OF AN INTRACELLULAR SYSTEM FOR RESPIRATION AND BIOELECTRIC POTENTIAL AT FLUX EQUILIBRIUM

GORDON MARSH

(WITH FOUR FIGURES)

Introduction

The oxidation-reduction theory of bioelectric currents formulated by LUND (14) has received much experimental confirmation (16, 17). The theory assumes the normal aerobic respiratory process in the living cell to give rise to the electromotively active materials, as follows:

$$\begin{aligned}&(a)\ X \rightarrow AH_2\\&(b)\ AH_2 + O \rightarrow A + H_2O\\&(c)\ A \rightarrow Y \rightarrow CO_2\end{aligned}$$

X is a precursor substance; AH_2 and A are the reductant and oxidant, respectively, of the electromotive material.

If we assume the dissociation $AH_2 \rightarrow A + 2H^+ + 2e$ we may write

$$[e] = \sqrt{\frac{K\,[AH_2]}{[H^+]^2\ [A]}}$$

where [e] is the electron concentration of the solution. The equation for the electromotive force developed at a locus (phase boundary) within the living cell may then be written:[1]

$$E_h = E_o - \frac{RT}{2F} \ln \frac{[A]}{[AH_2]}$$

where E_h is the potential referred to the hydrogen electrode, E_o a constant (containing $\frac{RT}{2F} \ln \frac{[H^+]^2}{K}$). The difference of potential across the living cell is assumed to consist of the difference between the oppositely oriented potentials of at least two loci, or

$$E_p = E_{h_1} - E_{h_2}$$

The E.M.F. measured across a tissue will be the algebraic sum of the polarity potentials of all the cells included in the measuring circuit, or

[1] The convention of sign is that of LEWIS and RANDALL (12, pp. 389, 402), which is the simple negative of the more commonly used European convention (20, p. 465). An increase in the ratio $\frac{[A]}{[AH_2]}$ increases the negative value of E_h. The European convention, however, has been followed in expressing the measured E.M.F. of cells and tissues. In consequence, the locus of high negative potential according to the equation is *measured* as the point of high positive potential. This has led to LUND's formal error of statement that the value of the ratio is smaller at that locus which is positive in the measuring circuit.

$$E = \Sigma E_p$$

For a given rate of oxygen uptake the ratio $\frac{[A]}{[AH_2]}$ will possess a constant value, a condition termed by LUND a flux equilibrium. The E.M.F. will vary with oxygen pressure and other factors influencing the rate of metabolism, although the variation is not defined precisely in LUND's formulation (in part due to the inclusion of an oxygen concentration term in the equation).

The present paper supplies a quantitative connection between the velocity of the oxidative reactions and the measured E.M.F. of a cell or tissue. Certain properties of the intracellular system thus emerge which are not otherwise obvious. The system also yields a satisfactory equation linking the rate of oxygen consumption to oxygen pressure.

At the risk of promoting a sterile controversy, it seems necessary to introduce the question of intracellular electrodes. The one objection urged against the oxidation-reduction theory of bioelectric phenomena is that metallic electrodes are absent from the cell (**2**). This argument is entirely *a priori*. It should be unnecessary to point out that the platinum (or other metal) electrode is *not* essential to the formulation of equations expressing the potential of an electrochemical system, nor to its manifestation (**6**, p. 378). "Metallic" conduction is not restricted to "metals," and a normal cell structure possessing the *essential properties* of an electrode is physically possible.

LUND's theory assumes the existence of such structures (or interfaces). Aside from its implications there is no evidence upon this point.[2] In view of the unanimity of the experimental support which the theory has received and its power to unite an extensive body of data into a coherent whole, the writer prefers to accept the existence of intracellular electrodes rather than to dismiss as irrelevant the evidence which LUND and his coworkers have amassed.

Relation of E_h to respiratory velocity at flux equilibrium

The steps in the oxidative reaction may be amended[3] to read:

[2] An attempt to provide direct evidence (**21**) has proved abortive. The color change reported at the borders of *Tradescantia* cells proved to be due primarily to retraction of the protoplast from the cell wall, not to a reversible pH change. The conclusion that conductors of the first class exist within the cell is unsupported (see also BLINKS, 3).

[3] LUND's equation (b) was not intended to specify the intervention of atomic oxygen. Consequently no great violence is done in altering the reaction to equation (2) above, while the mathematical treatment may be considerably simplified. The extra oxygen atom is here combined in A; for purposes of formulation the reaction might be written in any of a number of other ways. Until our knowledge of the specific chemical details of the respiratory process is greatly enhanced, the dynamics of the system cannot with profit be elaborated beyond some simple formulation like the present one.

$$X \rightleftarrows AH_2 \quad (1)$$
$$AH_2 + O_2 \rightarrow A + H_2O \quad (2)$$
$$A \rightarrow y \rightarrow CO_2 \quad (3)$$

Because of the existence of (a) "basal metabolism" and (b) an asymptote to the oxygen consumption:oxygen pressure curves (equation (15) below), we may write $[X] = C$. (C varies with the nutritional state, but appears constant for a given state.) The number of equivalents (per liter) of X transformed irreversibly in time *t* will then be $\frac{-dx}{dt} = k_1C = K_1$. At flux equilibrium the velocities of production and removal of AH_2 and A and the velocity of oxygen consumption will all equal K_1, unless respiration be primarily determined by rate of diffusion of oxygen, a condition which theoretical and experimental investigations indicate to be non-existent, or extremely rare (**11, 9**). Under these conditions the E.M.F. at a locus will vary with oxygen pressure but the rate of oxygen consumption will be constant. Since, experimentally, consumption does vary with pressure, $X \rightleftarrows AH_2$ must be reversible.

Let $[X] = C$. Then $k_1C =$ the tendency of X to form AH_2, and $k_2[AH_2] =$ the tendency of AH_2 to form X. The number of equivalents (per liter) of X changing to AH_2 in time *t* will be:

$$\frac{-dx}{dt} = k_1C - k_2[AH_2]$$

This is also the rate of production of AH_2 due to reaction (1), or

$$\frac{d[AH_2]}{dt} = k_1C - k_2[AH_2] \quad (4)$$

The rate of disappearance of AH_2 according to reaction (2) may be written

$$\frac{-d[AH_2]}{dt} = k_3P_c[AH_2] \quad (5)$$

where k_3 is the velocity constant of reaction (2) and P_c is the flux equilibrium oxygen tension at a locus within the cell.

At flux equilibrium (4) =(5), whence

$$[AH_2] = \frac{k_1C}{k_2 + k_3P_c} \quad (6)$$

The rate of disappearance of AH_2 equals the rate of production of A. Combining (6) and (5)

$$\frac{d[A]}{dt} = \frac{k_3k_1CP_c}{k_2 + k_3P_c} \quad (7)$$

The rate of disappearance of A due to reaction (3) is then

$$\frac{-d[A]}{dt} = k_4[A] \quad (8)$$

where k_4 is the velocity constant of reaction (3). As before, at flux equilibrium (7) = (8), and

$$[A] = \frac{k_3 k_1 C P_c}{k_4 (k_2 + k_3 P_c)} \tag{9}$$

The ratio of concentrations of oxidant and reductant at a locus (at flux equilibrium) will then be

$$\frac{[A]}{[AH_2]} = \frac{k_3 P_c}{k_4} \tag{10}$$

and the electromotive force developed at a locus becomes

$$E_h = E'_o - \frac{RT}{2F} \ln P_c \tag{11}$$

$\frac{-RT}{2F} \ln \frac{k_3}{k_4}$ is included in the constant E'_o.

P_c may be expressed as a function of the velocity constants of the reactions and the oxygen pressure of the medium surrounding the living cell. We may write the rate of diffusion of oxygen into a locus, in accordance with Fick's law, as

$$\frac{do}{dt} = k_o (P - P_c) \tag{12}$$

where P is the oxygen tension of the medium in contact with the cell and k_o is a diffusion constant for oxygen. The rate of oxygen consumption at a locus $\left(\frac{-do}{dt}\right)$ may be expressed by (7). At flux equilibrium the rate of diffusion equals the rate of consumption; equating (7) and (12) we obtain the expression

$$k_o k_3 P_c^2 + (k_3 k_1 C + k_o k_2 - k_o k_3 P) P_c - k_o k_2 P = 0$$

whose roots are $P_c =$

$$\frac{k_o k_3 P - k_3 k_1 C - k_o k_2 \pm \sqrt{(k_1 k_3 C + k_o k_2)^2 + P^2 k_o^2 k_3^2 - 2P(k_o k_1 k_3^2 C - k_o^2 k_2 k_3)}}{2 k_o k_3}$$

This may be simplified by writing

$$\frac{k_1 k_3 C + k_o k_2}{k_o k_3} = a, \text{ and } \frac{k_1 k_3 C - k_o k_2}{k_o k_3} = b$$

The constants are all positive in sign as written, and not zero. When $P = 0$, $P_c = 0$, hence

$$\frac{-a \pm \sqrt{a^2}}{2} = 0$$

Only the positive root satisfies this condition. Therefore

$$P_c = \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \tag{13}$$

and

$$E_h = E'_o - \frac{RT}{2F} \ln \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \tag{14}$$

Figure 1 shows the variation of P_c with P for certain values of the con-

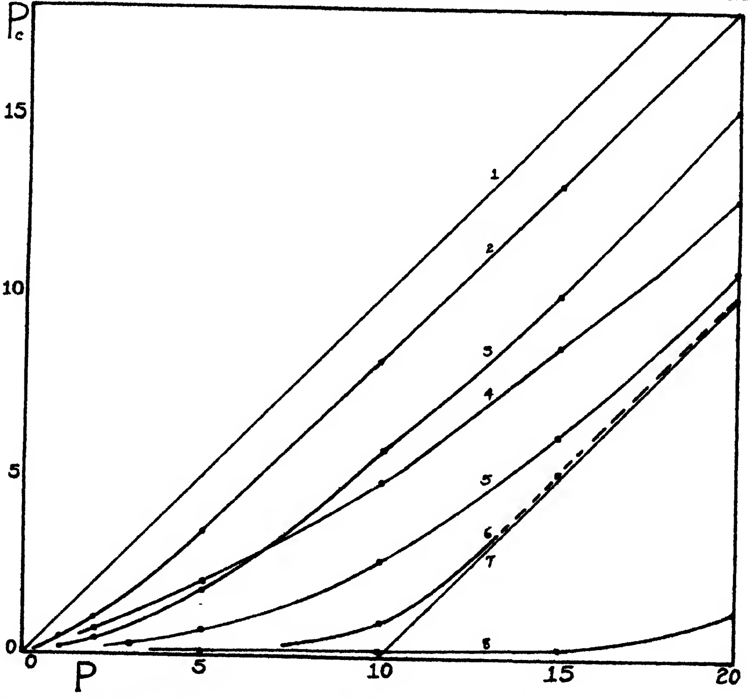

FIG. 1. P_c *vs.* P for the following values of the constants: (2) $a = 3$, $b = 1$; (3) $a = 6$, $b = 4$; (4) $a = 15$, $b = 5$; (5) $a = 11$, $b = 9$; (6) $a = 10.1$, $b = 9.9$; (8) $a = 20.1$, $b = 19.9$. (1) is the line $P_c = P$; (7) is the line $P_c = P - \frac{a+b}{2} = P - 10$, to which (4), (5), and (6) are asymptotic.

stants. $P_c = P$ at $P = 0$; from zero P_c increases with P to become asymptotic to $P_c = P - \frac{a+b}{2}$. The smaller the value of $a - b$ the more rapidly the curve approaches the asymptote, as may be seen in curves (4), (5), and (6). The reference line $P_c = P$, curve 1, is included in the figure to show how the flux equilibrium oxygen tension, P_c, differs from the external oxygen pressure, P, for different sets of values of the constants. The larger

the sum $a+b$ and the smaller the difference $a-b$ the more widely does P_c depart from P.

E_h, correspondingly, increases with increasing P (in harmony, it might be noted, with biological redox potential measurements). The locus in the living cell becomes, under this treatment, a modified oxygen electrode, and the ratio $\frac{[A]}{[AH_2]}$ cannot be combined with P (or [0]) in the equation for E_h, as done by LUND (**16**, p. 244).

Electrical polarity of the cell, E_p

The polarity potential of a cell will evidently be

$$E_p = \frac{-RT}{2F} \ln \frac{P_{c_1}}{P_{c_2}} = \frac{-RT}{2F} \ln r'$$

$$= \frac{-RT}{2F} \ln \frac{P - a_1 + \sqrt{a^2 + P^2 - 2Pb_1}}{P - a_2 + \sqrt{a_2^2 + P^2 - 2Pb_2}}$$

where the subscript 1 designates the locus of high positive potential in the measuring circuit. (The negative sign of E_p comes from the convention used, as explained above.)

Variation of r′ with P for four sets of arbitrary values[4] of the velocity constants is shown in figure 2. r′ increases from 1 at $P=0$, passes through a maximum, and again decreases to 1 as P becomes indefinitely large. E_p correspondingly would increase from 0 at $P=0$, pass through a maximum, and again return to 0. Figure 1 illustrates why this must be so. With the difference between the two loci determined by k_1 (thus avoiding intersection of the P_c curves), equal increments of P cause a relatively greater increase in P_c for the positive locus (as measured externally) in the lower portions of the curves, and the ratio r′ will increase. As the curves approach their parallel asymptotes, equal increments of P produce approximately equal increments in both P_c's, and r′ decreases.

The position of the maximum on the P axis and the absolute value of the maximum are functions of the values of the constants. No general solution for either position or value has been found. Differentiating r′ with respect to P yields an equation which is too involved to admit of solution by ordinary methods. It can only be demonstrated that substitution of appropriate values for the velocity constants furnishes numerical results which are in harmony with the experimental facts.

For the ascending limb of the curve the polarity potential increases with increasing P. The position of the maximum for systems whose variation

[4] For purposes of illustration it is assumed that the difference between two loci is solely determined by the velocity constant k_1, which is smaller at the locus of greater E_h. No significance is to be attached to such a choice of constants.

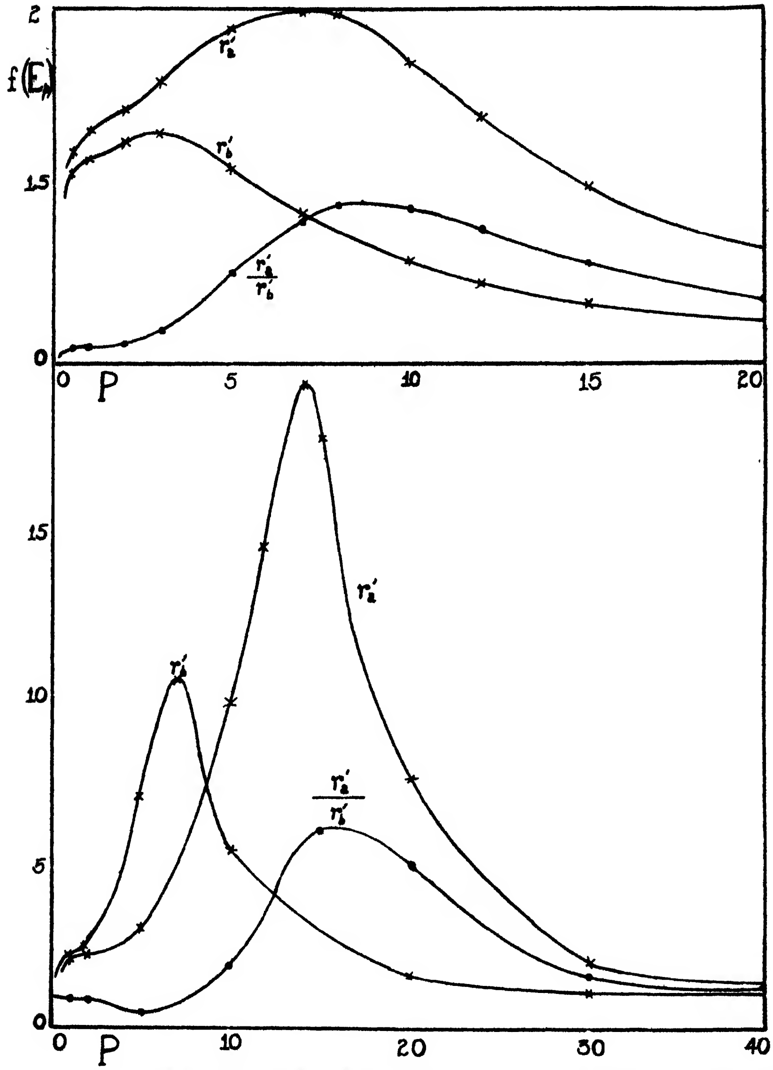

FIG. 2. Variables of the equations for E_p and E plotted against oxygen pressure. $r'_a = f(E_p)$ for apical cell; $r'_b = f(E_p)$ for basal cell. $r'_a/r'_b = f(E)$, the E.M.F. being oppositely oriented in the two cells. B shows reversal of apico-basal polarity at low values of P.

with P has thus far been investigated (frog skin, **15**, **8**; onion root, **22**) evidently lies outside the range of oxygen pressures used.

If the difference between the two loci giving rise to E_p involves one or more constants in addition to k_1 and C, the P_c curves may intersect (curves (3) and (4), fig. 1). Let P_{c_1} follow curve (3) and P_{c_2} follow curve (4).

E_p for such a cell would show, in addition to the behavior just described, complete reversal of polarity below $P = 6.5$ (approximate). The critical pressure for reversal, or intersection of the curves, would be determined by the magnitudes of the constants.

Such a reversal of polarity has recently been found for the apical 1.5 mm. of the onion root tip exposed to H_2 gas (**22**). The potential gradient of this region of the root is unidirectional as a rule. If basally oriented regions existed the reversal may have been of the type discussed in the following section.

Measured E.M.F. of a tissue or cell group

The E.M.F. measured across a tissue may evidently be (1) the sum of the elements of a unidirectional potential gradient. In this case the variation of E with P will not differ fundamentally from the variation of E_p with P. Or (2) E may be the algebraic sum of the elements forming two (or more) oppositely directed potential gradients, *e.g.*, the axially growing organs or organisms such as *Obelia* stem, roots and shoots of plants, etc. Analysis of the behavior of E for this type of tissue leads to a relation between respiratory rate and the polarity potential which gives a theoretical foundation to a number of observed facts.

In tissues and organs possessing apico-basal polarity the apex is electropositive to the base in the external circuit. The rate of oxygen consumption per unit mass is higher for apical than for basal tissues, and LUND (**16**) has demonstrated unequivocally for *Obelia* (and later work on the onion root) that this difference is due to the presence of a higher concentration of oxidizable material (AH_2) in the apical cells. These facts suggest that the magnitude of the electrical polarity of a cell or tissue is a direct function of its oxygen consumption rate (at constant oxygen pressure), where the differences in oxygen uptake between cells are caused by differences in value of their respective constants, leading to differences in flux equilibrium level of concentration of AH_2.[5] The properties of the system as outlined above are such as to yield this result.

The relation may be demonstrated as follows: Assume, as before, the difference between two loci in the cell to be determined by k_1. Let the differences in level of $[AH_2]$ (between cells) be caused by variation in C. For convenience of handling we may write o′ for $\frac{-do}{dt}$ at a given locus. Then from (12) and (5)

$$o' = k_0P - k_0P_c = k_8P_c[AH_2]$$

[5] The conclusion of LUND and MOORMAN (**19**) that the potentials of twelve frog skins bore no obvious relation to their rate of oxygen consumption offers no conflict to this view, if only upon statistical grounds. In fact their data suggest that for a large number of skins such a relation might appear.

or

$$P_c = \frac{k_o P}{k_o + k_3[AH_2]}$$

Substituting into (5) we obtain

$$o' = \frac{k_o P k_3[AH_2]}{k_o + k_3[AH_2]}$$

At any fixed and finite value of P, $[AH_2]$ will be determined by C, and

$$\lim_{C \to 0} o' = 0 \qquad \text{and} \qquad \lim_{C \to \infty} o' = k_o P$$

From (12)

$$P_c = \frac{k_o P - o'}{k_o}$$

and

$$r' = \frac{k_o P - o'_1}{k_o P - o'_2}$$

where the subscripts 1 and 2 designate respectively the positive and negative loci as measured externally. Then

$$\lim_{C \to 0} r' = 1 \qquad \text{and} \qquad \lim_{C \to \infty} r' = 1$$

For $0 < P < \infty$, $o'_1 < o'_2$ (equation 15), hence r' varies for fixed P and changing C in the same way as for fixed C and changing P (fig. 2), and E_p rises from 0, passes through a maximum, and again descends to 0 as C increases.

The rate of oxygen consumption of the cell will increase with C. Under these conditions, and for the ascending limb of the E_p *vs.* C curve, the cell having the higher oxygen consumption will possess the larger E_p, and this will be true for all values of P between 0 and ∞. Thus the net electropositivity of the apex of polar tissues over the oppositely oriented basal regions is susceptible of explanation.

This relation carries the implication that the state of nutrition of a tissue determines the magnitude of its measured E.M.F., for it is well known that the nutritional state affects the rate of oxygen uptake. Direct experimental proof has recently been presented by LUND (**18**), who showed that skins from frogs fed on liver gave potentials 83 per cent. higher (and oxygen consumption 28 per cent. greater) than those from starved animals. This is supported by observations that captivity results in lowered potential (**5**, p. 362; **8**, p. 381). The clearest indication of a connection between magnitude of potential and concentration of oxidizable material exists in the phenomenon of "rebound" or "overshooting," a temporary increase in P.D. when P is changed from a low to a high value (**15**, **8**, **22**). The corresponding over-

shooting of oxygen consumption rate is of general occurrence and is evidently due to accumulation of AH_2.

The "apical" cell will also show a relatively greater increase in E_p with P. For simplicity let the E.M.F. represent the algebraic sum of the oppositely oriented polarity potentials of an apical and a basal cell. Then

$$E = \Sigma E_p = \frac{-RT}{2F} \ln \frac{r'_a}{r'_b}$$

The subscripts a and b respectively designate the apical and basal cells. The difference between two loci is determined by the constant k_1 and this difference is the same for the basal as for the apical cell. The apical cell is assumed to have the higher value of C. The variation of $\frac{r'_a}{r'_b}$ with P is plotted in figure 2, using the values of the constants given in table I. $\frac{r'_a}{r'_b}$

TABLE I

CONSTANTS OF THE CURVES IN FIGURE 2

	2A: $(k_1)_1 = 3$, $(k_1)_2 = 5$		2B: $(k_1)_1 = 5$, $(k_1)_2 = 10$	
	APICAL CELL (r'_a)	BASAL CELL (r'_b)	APICAL CELL (r'_a)	BASAL CELL (r'_b)
C	2	1	2	1
a_1	7	4	10.1	5.1
b_1	5	2	9.9	4.9
a_2	11	6	20.1	10.1
b_2	9	4	19.9	9.9

increases from 1 at $P = 0$, passes through a maximum, and returns to 1 at $P =$ indefinitely large. The E.M.F. would increase from 0, pass through the maximum, and again return to 0. The curve is similar in form to r' *vs.* P and the position of the maximum lies slightly beyond that for the r'_a curve. The rate of oxygen consumption of the apical cell in both cases is greater than that for the basal cell (calculated by equation 15 below). For the ascending limbs of the curves, the system satisfies the observed dependence of apico-basal polarity upon P.

Two cases are theoretically possible for the ascending limb. In figure 2A the apical cell is everywhere positive to the basal save at $P = 0$ and $P = \infty$. When the value of the ratio $\frac{k_2}{k_3}$ is small compared with the value of $\frac{k_1 C}{k_0}$, however, the tissue will show an inverted polarity at low values of the oxygen

pressure, as appears in figure 2B. Reversal of tissue polarity under these conditions is due to the fact that E_p increases more rapidly in the vicinity of $P=0$ for the basal cell than for the apical.

Velocity of cell oxidation and oxygen pressure

The treatment given above yields a theoretical equation linking the velocity of cell oxidation to the oxygen pressure of the medium.[6] The rate of oxygen uptake at a locus is expressed by equation (7)

$$\frac{-do}{dt}=\frac{k_1Ck_3P_c}{k_2+k_3P_c}$$

Upon substituting the value of P_c from (13) we obtain

$$\frac{-do}{dt}=\frac{k_1Ck_3(P-a+\sqrt{a^2+P^2-2Pb})}{2k_2+k_3(P-a+\sqrt{a^2+P^2-2Pb})}$$

Dividing the numerator and denominator by k_3 and performing the substitution $\frac{2k_2}{k_3}=a-b$, gives

$$\frac{-do}{dt}=\frac{k_1C(P-a+\sqrt{a^2+P^2-2Pb})}{(P-b+\sqrt{a^2+P^2-2Pb})} \qquad (15)$$

Equation (15) is plotted against P in figure 3 for six sets of values of the constants. $\frac{-do}{dt}=0$ at $P=0$; it increases rapidly at first, then more slowly, and approaches k_1C as a limit as P becomes indefinitely large.

The measured respiratory rate of a tissue or cell will be the sum of the $\frac{-do}{dt}$'s for all the loci. For simplicity we may write[7]

$$0'=\Sigma\frac{-do}{dt}$$

The characteristics of the curve $0'$ *vs.* P will be similar to those of the individual $\frac{-do}{dt}$ curves, and the curve will approach Σk_1C as a limit as P increases. This may be seen in figure 3, where curve (1) is obtained by adding together the points on curves (2), (3), and (4). The summation curve will follow the course of an equation identical in form with equation (15), or

$$0'=\frac{\Sigma k_1C(P-a'+\sqrt{a'^2+P^2-2Pb'})}{P-b'+\sqrt{a'^2+P^2-2Pb'}} \qquad (16)$$

[6] For a recent review see TANG (24).

[7] $0'$ is introduced to avoid possible confusion of the more generally used A with the same symbol for the oxidant of the electromotively active material. $0'_m$ corresponds to the function A/A_o.

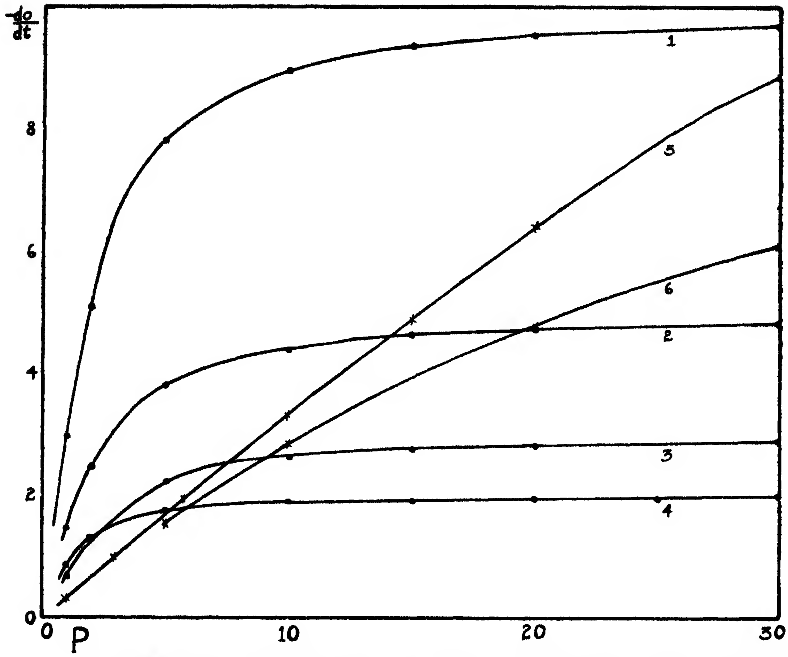

FIG. 3. Rate of oxygen consumption *vs.* oxygen pressure for the following values of the constants: (2) $k_1C = 5$, $a = 3$, $b = 1$; (3) $k_1C = 3$, $a = 4$, $b = 2$; (4) $k_1C = 2$, $a = 2$, $b = 1$; (1), the summation curve of (2), (3), and (4) is fitted by the same general equation; values of the constants are $\Sigma k_1C = 10$, $a' = 2.98$, $b' = 1.167$. (5) shows a nearly linear relation between consumption and pressure, $k_1C = 10$, $a = 30$, $b = 29$. (6) $k_1C = 10$, $a = 30$, $b = 5$.

a' and b' will lie within the extremes of the values of a and b respectively for the different loci. No general expression has been obtainable for determining their value.

Σk_1C may be eliminated in the following manner:

$$\text{Let } 0'_m = \frac{0' \text{ at P}}{0' \text{ at its maximum value}} = \frac{0' \text{ at P}}{\Sigma k_1C}.$$

Then,

$$0'_m = \frac{P - a' + \sqrt{a'^2 + P^2 - 2Pb'}}{P - b' + \sqrt{a'^2 + P^2 - 2Pb'}} \tag{17}$$

The behavior of 0_m *vs.* P for different values of a' and b' may be inferred from figures 3 and 4. The larger the value of a' the more slowly does the curve approach its asymptote. For a given value of a' the curve approaches its asymptote rapidly when $a' - b'$ is small, slowly when $a' - b'$ is large. With a' and b' very large and $a' - b'$ very small, the rate of oxygen consumption will appear linear over even a considerable range of P. In describing

experimental results of the linear type equation (16) would be employed, since the maximum rate of oxygen consumption is not determined.

Equation (17) has been applied to the majority of the data to be found in the literature, and in most cases yields a satisfactory fit. Space limitations prevent complete analysis of all available data; enough only is presented to illustrate the advantages and weaknesses of the equation as a description of experimental results.

Six sets of data are plotted in figure 4. The curves represent the course

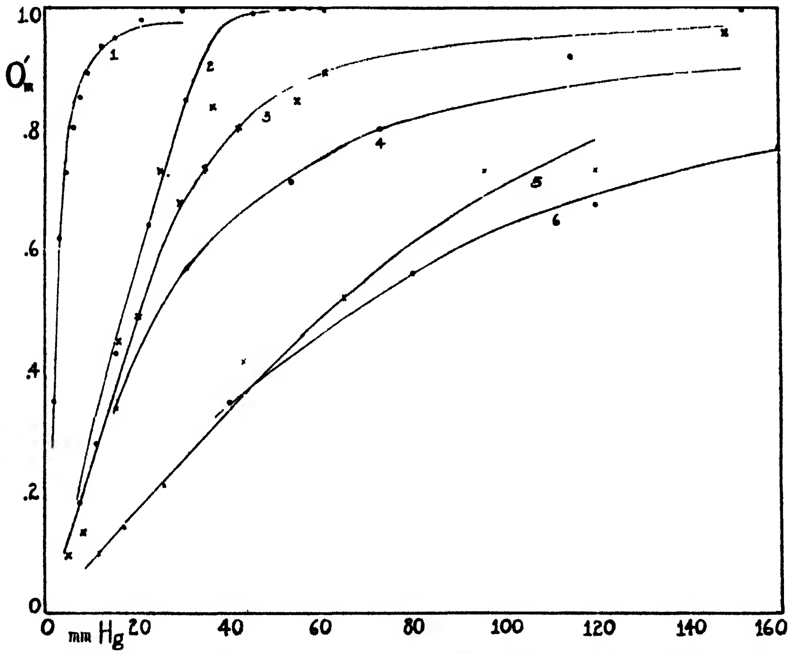

FIG. 4. Rate of oxygen consumption *vs.* oxygen pressure for six sets of published data: (1) SHOUP (23) luminous bacteria, $a' = 4$, $b' = 2.7$; (2) TANG and GERARD (25), fertilized *Arbacia* eggs, $a' = 35.4$, $b' = 35.26$; (3) LUND (13), *Planaria agilis*, $a' = 35.5$, $b' = 27.5$; (4) MEYERHOF (GERARD, 9, p. 267), NO_2 bacteria, $a' = 37.2$, $b' = 8.41$; (5) AMBERSON, MAYERSON, and SCOTT (1), *Homarus*, $a' = 113$, $b' = 90$; (6) THUNBERG (26), *Limax*, $a' = 93.6$, $b' = 17$. The curves follow the calculated values; observed values indicated by crosses or dots.

of equation (17); experimental points appear as crosses or dots. An arbitrary maximum was estimated for curve (5). For curves (2) and (6) the correspondence of observed and calculated values is nearly complete. SHOUP'S (23) results for luminous bacteria, curve (1), reach the maximum somewhat earlier than the calculated curve. Curves (3) and (5) show good correspondence except for the scattering of the experimental points. An incomplete fit is afforded the data of MEYERHOF on NO_3 bacteria (table in 9,

p. 267), shown in curve (4). Calculated and observed values correspond almost exactly until P reaches about 80 mm. Hg, beyond which the observed points deviate, reaching $O_m = 1$ in advance of the theoretical curve. Similarly data by TANG on unfertilized *Arbacia* eggs (**9**, p. 266) can be accurately fitted save for the upper three values. Determination of the point of 100 per cent. respiration is not always easy, and a moderate error may considerably alter the shape of the resulting O_m *vs.* P curve.

The equation will not follow data which show an upward concavity when plotted as above, *e.g.*, those of AMBERSON, MAYERSON, and SCOTT (**1**) for *Nereis.* In the modified form, however, it will describe the linear relation between O′ and P found by HALL (**10**) for the toadfish. Reasonable correspondence is found for other data (scup and puffer, **10**; fertilized *Arbacia* eggs, **25**; frog's skin, **15**; termite, **7**; and others). An almost point to point fit is given the curve for NO_2 bacteria, MEYERHOF (table in **9**) and to data by BODINE (**4**) on embryos of *Melanoplus differentialis.*

The theoretical equations relating oxygen consumption to oxygen pressure through the effect of oxygen upon the reaction velocity of the oxidative process are summarized by TANG (**24**). All take the hyperbolic form $A = \frac{P}{K_1 + K_2P}$ where A is the oxygen consumption rate, P the oxygen pressure, and K_1 and K_2 are constants. GERARD (**9**), using the equation in the form

$$\frac{A}{A_0} = \frac{[O_2]}{K + [O_2]}$$

found K only roughly constant, if at all, for data by SHOUP, TANG, and MEYERHOF. Except for TANG's data the variation in K was greatest in the lower range of P. The graphical application of the equation to twenty-four sets of data by TANG (**24**) likewise is least successful at small values of P. (A scale for the ordinate in TANG's figures would make it possible to judge how well the equation really fits.)

In the "hyperbolic" equation P is interpreted to be the oxygen pressure of the medium, although upon the underlying assumptions it must be the intracellular oxygen pressure. The equation is identical with (7) above, and is valid only when P_c/P approaches 1. This will occur as $P \rightarrow 0$ and as $P \rightarrow \infty$; it will be true for all values of P *only* for cells in which $\frac{a+b}{2}$ is very small. It would be expected, therefore, that for small values of P the hyperbolic equation would show a poor fit for numerous experimental data, as GERARD and TANG apparently have found.

The estimation of the relative applicability of similar mathematical formulas to experimental results is uncertain. Nevertheless, because of its competency to follow experimental values in the lower range of P where

the hyperbolic equation tends to deviate, and because of the somewhat sounder theoretical foundation, the writer is of the opinion that the equation developed in this paper will prove more generally valid.

Finally it may be remarked that the derivation of a valid oxygen consumption: oxygen pressure relation upon the basis of the formulation of the reacting system for E.M.F. fulfills a necessary condition for the oxidation-reduction theory of bioelectric currents.

Summary

A quantitative connection is derived between the velocity of respiration and inherent cellular E.M.F. upon the basis of the amended steps in the oxidative reaction proposed by LUND. The system shows the following properties:

1. Direct dependence of the potential at a locus upon oxygen pressure.
2. Dependence of the electrical polarity of a cell (difference between two or more loci) upon oxygen pressure.
3. Direct dependence of the magnitude of cell polarity upon the concentration of the oxidizable material, *i.e.*, upon rate of oxygen uptake at constant oxygen pressure.
4. Relatively greater increase in cell polarity with increased oxygen pressure for the cell possessing the higher concentration of the oxidizable material, *i.e.*, dependence of apico-basal polarity upon oxygen pressure.
5. Reversal of cell or tissue polarity at low oxygen pressures under appropriate conditions.

An oxygen consumption-oxygen pressure equation is obtained which satisfactorily describes existing data.

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PHYSIOLOGICAL PROBLEMS CONNECTED WITH THE USE OF SODIUM CHLORATE IN WEED CONTROL

A. S. CRAFTS

Introduction

The use of sodium chlorate for controlling weeds has become a general agricultural practice within the past decade. The attendant experimental work has produced a voluminous literature and has brought into prominence many problems of a purely physiological nature. Since progress in the experimental work on chlorate apparently depends upon properly comprehending the mechanics of its distribution and absorption, work on these phases would have an immediate value. This paper presents several problems for consideration by plant physiologists and persons experimenting with weed control and discusses them in the light of our present knowledge.

ÅSLANDER (1) reported in 1926 that chlorates would kill plants by absorption from the soil, and in 1928 (2) he discussed critically the problem of weed control by this method. Meanwhile, however, the use of sodium chlorate solution as a spray (**11, 16**) claimed the attention of weed workers; and in the popularity of this method, ÅSLANDER's results were apparently overlooked. Most workers showed by their recommendations that they pictured the toxic action as taking effect through the plant after absorption of the chlorate by the leaves. Stickers, spreaders, various pressures, types of nozzles, and volume-concentration relationships were studied as they affected coverage of the foliage. Control of the hydrogen ion concentration of the spray solution, the inclusion of hygroscopic agents, and spraying methods in general were considered in attempts to improve the technique and avoid the erratic results so often encountered in the field.

Of twenty-nine publications on the use of chlorates that had appeared by the end of 1931, only three (**1, 2, 17**) emphasized the possibilities of the soil-application method. In 1931, LOOMIS, BISSEY, and SMITH, pointing out the advantages of this method, suggested that the action of sodium chlorate, as used in regions of summer rains, resulted largely from root absorption of chemical leached into the soil. Since then MUENSCHER (**20**) has pointed out the practical nature of the soil method, while LOOMIS and others (**18**) have given further experimental evidence of its feasibility.

Meanwhile there had been developed in California a method for treating certain deep-rooted perennials with acid arsenical spray (**4, 5, 8, 15**). Its success depends upon translocation of the toxicant within the vascular system of the plant. The question naturally arises, will this same mechanism

carry chlorate in the plant and, if so, to what extent does it explain the results obtained in the common use of chlorates?

As the writer has recently reported (**6**), chlorate may be moved through plants and may kill the roots to considerable depths as a result of leaf absorption and translocation within the xylem. The effects of a given treatment are limited by the same factors that govern penetration and root killing by the acid arsenical. When conditions are right, any application of chlorate solution to the foliage will result in some absorption and downward movement within the plant. The extent of injury will depend upon how nearly the requirements for successful treatment by this method are met.

Besides these two distinct mechanisms available for use in treating deep-rooted weeds, there are several interesting physiological responses by the plant to both lethal and sublethal doses of chlorate. The various effects that chlorates may have on plants will therefore be listed and discussed. Wherever possible, their relation to weed-control practice will be pointed out.

Physiological action of chlorates

There are, apparently, four fundamentally different ways in which chlorates may affect plants:

1. When a chlorate solution is applied to leaves, ions of the salt diffuse through the cuticle and come into contact with the protoplasm of living cells (**18, 5**). Although the coefficient of permeability of plant cells to chlorate ions is low (**23**), when they attain a lethal concentration they enter the plant cell, causing injury and eventually death. The exact mechanism by which this result is accomplished is not known. It has been suggested that the high oxidizing potential of sodium chlorate (**22**), the presence of pentavalent chlorine (**22**), and the complete oxidation of respiratory chromogens (**12**) may be involved; but no experimental evidence has been presented to support these ideas. Contrary to popular notion (**14, 22, 23, 24, 26**), sunlight or ultraviolet light is not essential to this process, which will take place in the dark (**17**); and open stomata (**19**) are not necessary, the chemical being fully toxic when applied to the ventral surfaces of hypostomatic leaves (**5, 18**). The solutions commonly applied as sprays in no way compare in concentration with those generally used in physiological experiments (**23**). They usually become saturated and therefore strongly plasmolytic soon after application, a steep gradient in concentration developing across the cuticle layers.

2. When chlorate ions exist in the plant in sublethal concentration, there is a typical response, characterized by a chlorotic, stunted condition of any growth that occurs, a reduction of the starch reserves (**16**), decreased catalase activity (**21**), and increased susceptibility to frost injury (**16**). These

symptoms may precede death of the tissues if conditions favor the continued uptake and accumulation of chlorate. Where additional chlorate is not available, the plant may recover and show no permanent effects.

3. Under certain limited conditions, a concentrated chlorate solution applied to the leaves of plants may penetrate the cuticle, plasmolyze and kill the mesophyll tissues, enter the xylem, and be carried down into the roots (**7**). The conditions essential to this action have been discussed in connection with acid arsenical sprays (**4, 5, 8, 15**). Experiments to be described indicate that the same response may occur with chlorates and will result in a rapid, deep killing of the root system.

4. Chlorates present in solution in the soil may be absorbed by roots, killing all parts in which they accumulate to a lethal concentration.

Although it has been intimated that chlorates may penetrate the leaves and be translocated through the phloem (**11, 22**), this possibility seems rather remote in view of the prevailing concepts of phloem tissues (**3, 9**). Movement of chlorate through a system so dependent upon the functioning of living cells is hard to imagine.

Under different conditions of treatment the four effects listed may occur in almost any combination, singly or together. Rarely in the field are less than two concerned; and often all four may be in evidence during the year. Naturally, therefore, results of plot tests have been difficult to interpret, and recommendations from different localities have been inconsistent and confusing.

Before discussing these four responses of plants to chlorate in greater detail, it would be well to consider briefly the inherent differences in plants with respect to injury in general and also with respect to injury by chlorates.

Many plants are easily killed. When cut off at the ground level, they fail to recover and die with no further treatment. Other plants will resprout from the stump or crown, and still others may regenerate from stem, rhizome, or root tissue and are eradicated only when all vestiges of the root are killed or removed from a suitable environment. In treating a variety of plants with a toxic substance like chlorate, one is sure to find differences in the degree of control; and unless he has previously determined their response to mechanical injury he cannot accurately interpret his results.

Furthermore, plants vary widely in their susceptibility to injury by chlorates. Although this fact has been pointed out many times (**6, 10, 13, 28, 29**), it will bear repeating because it is a striking phenomenon, vitally involved in the determination of dosages. Until one has determined in an empirical way the susceptibility of a particular species, he cannot well prescribe the dosage necessary for its control in the field.

Killing of plant tissue by chlorate

It has been pointed out that whenever chlorate ions come in contact with plant cells in sufficient concentration, the tissue is killed. Though the mechanics of this killing process form an interesting field for speculation, the critical point, so far as weed control is concerned, is that only the presence of chlorate in lethal concentration is necessary for the death of the cells. Our principal problem is distribution of the toxicant. For the desired results, it must come into contact with the vital tissues.

Obviously, therefore, the technique of application must be related to the plant concerned. If the plant is easily killed, a thorough spraying of the foliage is sufficient. If it resprouts weakly, spraying of the regrowth may effect a kill. On the other hand, if the plant regenerates strongly from the roots, killing of the tops is a small part of the problem, and spraying is effective only as it may lead to leaf absorption and rapid killing by xylem transport as described in (3), page 701, or as it distributes the chemical for leaching and absorption from the soil. Apparently the killing of the cells by chlorate is largely independent of the environment and is a matter only of concentration and cell activity.

Physiological responses of plant cells to chlorate

The effects of chlorate upon plants have considerable interest for workers in plant physiology. Concentrations in the tissues near or below the lethal point cause reactions that are apparently peculiar to chlorate alone. LATSHAW and ZAHNLEY (**16**) pointed out the great reduction in starch reserves of roots after chlorate treatments. The writer has observed the same phenomenon not only in sprayed plants but in those which have absorbed chlorate from the soil. It has been seen in stems of plants containing a sublethal concentration and is undoubtedly present in all tops showing the stunted chlorotic growth that commonly follows dusting with Atlacide, or soil applications. Lowered catalase activity (**21**) and susceptibility to frost injury (**16**) accompany this condition; and, taken together, these responses indicate a lowered vitality in the presence of chlorate ions.

As has been mentioned, when conditions favor continued absorption of chlorate, this condition of lowered vitality is accentuated, so that the tissues finally die. With lowering chlorate concentration, however, they may recover and show no permanent injury. From the standpoint of weed control this response of plants to chlorates is of minor importance, being useful only as an indicator of the presence of the chemical.

Killing of plants with chlorate following leaf absorption and transport to the roots through the xylem

Having already been described, the action of this mechanism (**4, 5, 8, 15**) requires no further comment. That it will respond to chlorate sprays has

been demonstrated (6). Its possibilities in weed control, however, are of interest; and its relation to results obtained by present methods warrants comment.

Loomis and others (18) have shown that chlorate ions will enter and move through the xylem of plants. Their results are not surprising, for any solution of molecularly dissolved substance that does not undergo chemical change within the xylem will displace the xylem sap and follow the transpiration stream wherever the gradients in pressure may cause it to flow. The critical point is, will chlorates kill the leaf cells and, rendering them permeable, penetrate the xylem and be carried deep into the roots, causing their death? Recent developments (4) indicate that this method has practical possibilities, after all, and that chlorates might find a logical use in certain cases.

Although some evidence has been presented previously (6) and although this paper does not aim primarily to submit extended experimental results,

TABLE I

Effect of time of day, acidity, and concentration upon the root killing of morning-glory plants by sodium chlorate

Plot no.	$NaClO_3$ per sq. rod	H_2SO_4 per sq. rod	Time of application	Plants re-sprouting October 8, 1931
	lb.	*lb.*	P.M. Aug. 20, 1931	%
1	1		4:00	90
2	1		7:00	90
3	2		4:30	90
4	2		7:30	40
5	3		5:00	80
6	3		8:00	10
			P.M. Aug. 21, 1931	
7	1		4:00	90
8	1		7:00	90
9	2		4:30	90
10	2		7:30	30
11	3		5:00	80
12	3		8:00	5
			P.M. Aug. 22, 1931	
13	1	1.25	4:00	10
14	1	1.25	7:00	10
15	2	1.25	4:30	40
16	2	1.25	7:30	10
17	3	1.25	5:00	10
18	3	1.25	8:00	5

the data on a few trials will be given to show the nature, possibilities, and limits of this method.

Table I presents the results of spraying a series of plots with sodium chlorate solutions in August, 1931. These applications were made during a period when the daytime temperatures reached 100° F. Though temperatures were somewhat lower toward evening, the relative humidity was low, and all sprays applied before sundown dried very rapidly on the leaves. The plots were in a young orchard that had been irrigated and disked late in June. A dense growth of morning-glory had matured and had lowered the soil moisture to a point approaching the permanent wilting percentage at the time the sprays were applied. Each plot was approximately 1 square rod in area, and the chemical was applied in 3 gallons of water. The foliage on all plots was killed within 48 hours.

As no rain fell between August 20 and October 8, 1931, the results at the latter date represent the action of chlorate on and through the plants. The plants on the plots showing low percentages of resprouting were killed to a depth of 3 feet or more. Glancing over the data, one observes that the more concentrated sprays were the more effective, that delaying the application until after sundown materially improved the results, and that sulphuric acid markedly increased the efficiency of the method.

Table II presents the results of some plot tests with sodium chlorate applied on September 28, 1931. Plots 4 to 6 and 10 to 12 in this series had been recently irrigated. The temperature at this date was lower and the relative humidity higher, so that penetration during the day was considerably better than in August. These results again show the effects of concentration of the solution and the value of adding acid to increase the rate of penetration. In addition they show the effect of soil moisture upon the action of the mechanism. Even though transpiration was high at this season, the plants in the moist soil did not have the high water deficit of the others, and distribution of the toxicant in the roots was less complete.

These experimental results are typical of a good many obtained during the work with chlorates. They indicate that this method has certain possibilities in areas where the plants deplete soil moisture rather thoroughly. In regions of frequent summer rains it would have no value.

In considering the practical possibilities of this method, one must note several points. First, it would probably never be more effective than the acid arsenical, and the chemicals will necessarily cost from three to five times as much. Second, the acid cannot be applied along with the chlorate, for the combination forms a strong oxidizing mixture that will ruin any machinery; it would have to be applied as a separate spray following the chlorate application. Finally, applications under the proper conditions would involve the fire hazards attending the use of chlorates.

TABLE II

EFFECTS OF APPLICATION RATE, SOIL MOISTURE, AND ACID UPON CHLORATE KILLING OF MORNING-GLORY

Plot no.	Date irrigated	Sprayed Sept. 28	$NaClO_3$ per sq. rod	H_2SO_4 per sq. rod	Resprouting Nov. 23, 1931
			lb.	*lb.*	%
1	Aug. 15	4:20 P.M.	1	....	20
2	Aug. 15	5:20	2	....	15
3	Aug. 15	3:45	3	....	5
4	Aug. 15	4:10	1	....	45
5	and	5:10	2	....	35
6	Sept. 24	3:15	3	....	40
7	Aug. 15	4:40	1	1.25	1
8	Aug. 15	5:00	2	1.25	0
9	Aug. 15	5:40	3	1.25	0
10	Aug. 15	4:30	1	1.25	25
11	and	4:50	2	1.25	25
12	Sept. 24	5:30	3	1.25	25

On the other hand, with due caution this method might be used in pasture areas where the acid arsenical would present a poison hazard. Also, where conditions are optimum for this type of spray, the experimental results indicate that the chlorate dosage may be materially reduced. Furthermore, the application of sulphuric acid in a dosage providing a weight of the concentrated acid equal to that of the sodium chlorate will liberate chloric acid and reduce the chlorate ions reaching the soil so that little or no residual effect will be found. Sulphuric acid is best applied in a concentration of about 5 per cent. by weight or approximately one normal. Even this is corrosive to machinery and requires special acid-resistant equipment. Such equipment is available for using sulphuric acid as a spray on mustard in cereals.

Killing of plants with chlorates by absorption from the soil

It is apparent that treatment of deep-rooted weeds through the soil is a logical method. The regenerative organs themselves are directly affected by the chemical; with proper dosage and distribution there is little possibility of failure. The method is not subject to those diverse and difficultly controlled factors to which the foliar organs are exposed.

In the problem of dosage, species susceptibility and the effect of soil type on chlorate concentration are vital factors. The problem of species

susceptibility has been mentioned. Variations are extremely wide. TIMSON (25) describes a parasitic plant called "witch weed" that requires a maximum of only 80 gallons of spray containing 12 pounds of sodium chlorate per acre for control of a solid infestation. At the other extreme, chlorates have often been applied to hoary cress and other of the less susceptible species at rates of 6 and 8 pounds per square rod with only partial kills. Though these latter results may be due in part to improper methods, more susceptible species treated under identical conditions are often completely eradicated.

At present the only hope for determining the dosage to use on a particular species in the field is an empirical test under local conditions. Though the operator may be guided somewhat by results described in the literature, soil and climatic factors so affect the growth of plants that local experience is usually required.

The influence of soil type upon chlorate toxicity, having been introduced in another place (7), will be only touched upon here. Though toxicities may vary as widely as five times between soils of different types (7), no generalization can be offered now that will aid in their determination. Again empirical testing is indicated as the most promising method for studying this effect. It seems at present that, within a soil series, toxicity will run higher in the coarser grades. Among series, recent alluvial soils exhibit the lowest toxicity; old weathered soils the highest. Much more work is required, however, before these statements can be proved. The results of such testing at this station will appear as the work is continued.

The fixing of chlorate in a form available to plants is another soil property to be considered. As experiments have shown (7), certain soils are able to hold chlorates so that they do not move freely in the soil solution. In general the soils showing the lowest toxicity have the strongest fixing power, and in these the proper vertical distribution of chlorate within the soil is a problem. Under the conditions at Davis, morning-glory must be killed to a depth of at least 4 feet or it will resprout and survive. A lethal concentration of chlorate, therefore, must be provided throughout the top 4 feet of soil if the treatment is to be a success. In a Yolo silt loam, leaching experiments showed (6) that about 6 inches of water were required when the chlorate was applied to moist plots, and from 8 to 12 inches when dry plots were treated. In a heavier soil these requirements might well be doubled. Where the moisture came as rainfall with opportunity for evaporation between storms, even more water would be needed. This one factor probably explains many of the failures attending the use of chlorates in the arid regions of the west.

Soil moisture enters the problem again in the matter of chlorate decomposition. Where rains keep the soil wet during the hot summer season,

residual effects from chlorate treatments are of little importance. Where the top soil dries out during the summer, chlorate remains intact and may persist for three years (**6**) or more. Under these conditions leaching with irrigation water is the easiest method for ridding the land of chlorate (**6, 7**). Where irrigation is not available, chlorates should be used with considerable discretion on heavy soils.

The proper season for chlorate application has been much debated. Many publications recommend the blossoming stage of the plants, which usually occurs in early summer. Sometimes the same recommendation will state that the plants should be mature or *fully* mature for successful treatment. In central California morning-glory comes into blossom in April and May. On unirrigated land it reaches maturity, as denoted by ripening of seed and cessation of terminal growth, in June and July. On irrigated land it may not mature until October. Obviously, therefore, these recommendations cannot be followed in any one treatment.

Hulbert, Bristol, and Benjamin found in Idaho (**13**) that treatments from May to August were equally effective by the following summer. Willard (**27**), and Sampson and Parker (**24**) treated successfully during the spring; Åslander (**1, 2**), Muenscher (**20**), and the writer (**6**) have had excellent results from winter treatments. Stage of growth therefore would appear to have little effect in this response. Careful analysis, however, indicates that this is not altogether true. Loomis and others state: ". . . sodium chlorate dissolved in the soil water readily penetrates and kills the roots and rhizomes of either active or dormant plants" (**18**). Although this is undoubtedly true, absorption of chlorate by dormant roots is apparently slower than by active ones. The writer has noted many cases where plants have survived fall and winter applications only to weaken and die in the early summer. Spring and early summer treatments through the soil have been generally more successful than summer and fall applications except as the latter have provided a lethal concentration in the soil for absorption during the following spring. Midsummer applications have given the greatest success only in regions of summer rainfall where the chlorate is very soon made available for absorption through the roots.

The plant root goes through three definite stages during the year. In the spring and early summer it is in a vegetative state characterized by rapidity of growth, absorption of water and mineral nutrients, and depletion of organic food reserves. During the summer and fall it replenishes its food reserves; growth and absorption become slower. During the winter, growth and absorption are at a minimum, and organic foods are only slowly changed. From the standpoint of its physiology, the root should be most susceptible to chlorate injury during its vegetative stage in the spring. The discussion just presented indicates that this is probably true.

At least two important factors are involved in this increased susceptibility during the spring. Rapid absorption of water and mineral nutrients by the roots should favor absorption and accumulation of chlorate within the plant, and the low level of food reserves should make the roots vulnerable. On the other hand, the gradual killing of plants in the spring following fall and winter applications takes place generally where a given dosage has been leached to a considerable depth and the actual chlorate concentration at any point is low. With the depletion of soil moisture, the chlorate concentration increases generally throughout the soil mass. In addition, because of the water moving into the plant roots and the selective rejection of chlorate resulting from the low coefficient of permeability of protoplasm for these ions, the chemical must concentrate at the surface of the root. As the ions concentrate to the lethal point the roots become injured, increasing in permeability, and then chlorate enters the plant in much greater amounts than before. The result is the complete breakdown and death so often noted as the soil moisture runs low. The effective concentration at the absorbing surfaces must be much higher than if the soil moisture were maintained by irrigation or rainfall. That this factor of concentration within the soil is important is indicated by the number of recommendations that warn against irrigating after chlorate applications. The writer has used irrigation water (**6**) for attaining a vertical distribution of chlorate in the soil with considerable success. Apparently it is harmful only where it maintains a high moisture content that effectively dilutes the chlorate, or where it leaches the chlorate beyond the region of absorbing roots.

Discussion and summary

Chlorate, as a plant poison, seems to have unique properties. It acts slowly (**18**) compared with the heavy metals, seeming to enter the plant in low concentration, and gradually accumulates if the source of supply is maintained. Though arsenic, when present in the soil, affects principally the absorbing organs and has little primary effect upon the tops, chlorate apparently affects the whole plant at the same time and in essentially the same way. Seedlings that have germinated in soils containing large amounts of arsenic are often found to have their roots so injured that water is absorbed through dead tissues as through a wick. Plants in soils containing chlorates in just as injurious quantities have strong root systems. Here the plants may exhibit considerable growth and then dry up and die completely. Very high concentrations of chlorate in the soil will kill roots just as does arsenic.

The writer is reminded of some early experiments with arsenic and chlorates on morning-glory shoots. Excised shoots placed in dilute solutions of these two chemicals react characteristically. The young tender tips of the shoots in arsenic solutions turn black and droop within 16 hours, and

the older leaves die later. With the chlorate-treated shoots, the older leaves die first; the tips turn chlorotic but do not die, and many actually grow for several days, elongating to the extent of 10 to 20 centimeters. Apparently, arsenic is primarily a protoplasm poison and kills as it goes, entering young and old tissues alike. Though chlorate will also kill by contact when present in high concentrations, it seems to enter the plant more slowly when absorbed from the soil or when applied in solutions of low concentration and allowed to act by accumulation in the tissues. The plant dies under these circumstances, not because the protoplasm has been killed by direct reaction with the toxicant, but rather because this material so disturbs the metabolic processes that the plant can no longer function normally. The symptoms are systemic in nature, and apparently assimilation and utilization of foods as well as other vital functions are affected.

The discussion presented shows that there can be no universally successful method for using chlorates. With the extreme variations in susceptibility of species and the number of factors affecting toxicity and absorption, methods must be adapted to the conditions of the treatment, the operator taking every possible advantage of the situation at hand. Though summer rains provide an ideal means for distributing the chemical in the soil, insuring the success of spring and summer applications in the humid regions, other methods must be used in the more arid parts of the west. Directions provided by chemical companies and experiment stations for the use of chlorates should be adapted to the locality in which the chemical is to be used.

The successful use of chlorate is obviously more difficult in arid regions. In California, three methods have been proposed (6): (1) fall spraying where rapid absorption and root killing are followed by leaching and absorption from the soil; (2) a straight soil treatment during the winter; (3) spring soil treatment followed by proper irrigation. All three methods, however, are subject to differences in growth conditions and soil type, and the first two depend upon rainfall. They must be used, therefore, with utmost care.

All soil applications should aim to provide a toxic concentration of chlorate throughout a proper depth of soil for absorption during the spring vegetative season. Plants left undisturbed in such soils accumulate chlorate as the moisture decreases and grow weaker with the advancing season. Hoeing or weed cutting will eliminate the few plants that struggle along and sometimes survive this treatment (6). This should not be done, however, until the plants are severely affected. At least one complete season must be allowed for success by the chlorate method, and provision must be made for destroying such seedlings as appear in succeeding years.

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PHYSICAL AND CHEMICAL PROPERTIES OF THE SOLUBLE POLYSACCHARIDES IN SWEET CORN

M. W. Parker

(WITH ONE FIGURE)

Introduction

The endosperm of sweet corn, in contrast to that of most varieties of maize, contains a relatively high percentage of soluble polysaccharides, but very little is known concerning their physical and chemical properties. On the basis of the iodine color alone, Culpepper and Magoon (8) concluded that these water soluble polysaccharides are composed of both dextrins and soluble starch.

This investigation deals with the isolation and purification of the water soluble polysaccharides from green and mature Hopeland sweet corn kernels, and with their physical and chemical properties. These properties were compared with those of alpha and beta amylose from mature Hopeland sweet corn starch. The chief purpose of this comparison was to determine whether the soluble polysaccharides are true dextrins produced by starch hydrolysis or whether they are synthesized amyloses that have not been organized into starch grains.

Investigation

Extraction and purification of soluble polysaccharides

The green sweet corn was prepared for extraction of the soluble polysaccharides by splitting the kernels and scraping out the contents. The air dry mature corn was first soaked in 20 per cent. alcohol and the softened kernels were then ground through a Nixtamal mill. Enzymatic action was checked during the extraction by 1×10^{-4} N iodine (18).

These polysaccharides are soluble in water and in alcohol up to 30 per cent. by volume and are precipitated by alcohol above this concentration. Therefore 20 per cent. alcohol was used as the solvent during extraction and purification. Samples of the corn pulp were extracted twice with cold 20 per cent. alcohol. The first extraction was made soon after adding the alcohol while the second was made after shaking for one hour. The extracts were separated from the pulp by means of several thicknesses of cheesecloth. These extracts contained the soluble polysaccharides, starch, and various other substances. The starch was removed from the extracts by centrifuging and filtering. The soluble polysaccharides were then precipitated from the filtrate by increasing the alcoholic concentration to 60 per cent. by volume. The 60 per cent. alcohol was removed and the material was re-

dissolved in 20 per cent. alcohol. This solution was centrifuged and filtered in order to remove impurities. The process of dissolving in 20 per cent. alcohol and reprecipitating in 60 per cent. alcohol was repeated until no residue was deposited on centrifuging the 20 per cent. alcoholic solution and qualitative tests for proteins in the wash alcohol were negative. Final purification of the soluble polysaccharides was effected by dissolving in water, filtering, and subjecting the filtrate to electrodialysis. The apparatus (fig. 1) employed for electrodialysis is a modified form of the chamber used by

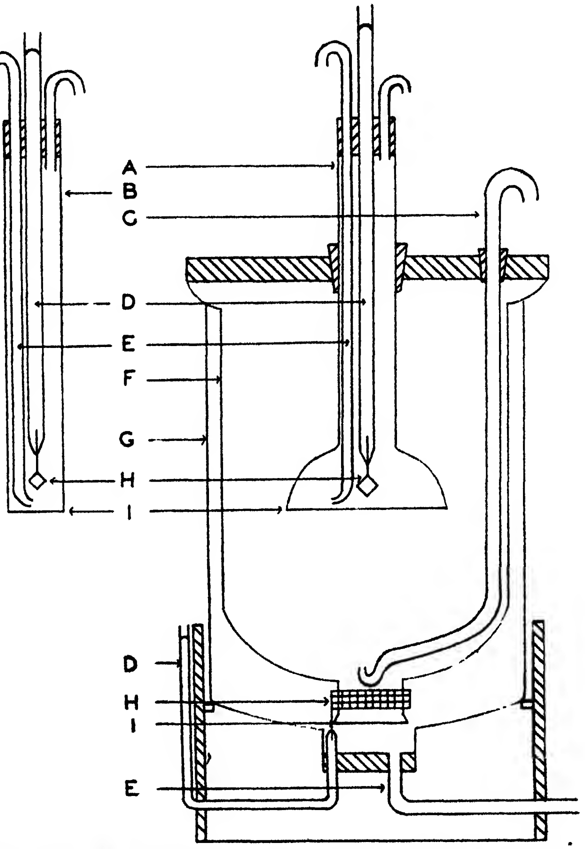

FIG. 1. Electrodialysis apparatus: *A*, improved negative electrode chamber; *B*, negative electrode chamber used until 1932; *C*, siphon for dialysis chamber; *D*, mercury connections for electrode; *E*, siphon for electrode chamber; *F*, dialysis chamber; *G*, positive electrode chamber; *H*, platinum electrodes; *I*, collodion membranes.

TAYLOR and IDDLES (23). The electrode chamber which was used for the purification of the samples taken in 1930 and 1931 is shown in figure 1 *B*; the electrode chamber which was adopted in 1932 is shown in figure 1 *A*. The new electrode chamber has a much larger membrane surface and allows

a larger volume of water to surround the electrode. This improvement hastened the separation and purification of the materials investigated. The membranes of the electrode chambers were of collodion supported on cheesecloth and were of such thickness that they allowed the free passage of inorganic ions while retaining the polysaccharides. A current of 250 volts d.c. was passed through the solutions until they were free of unbound inorganic ions. This point was detected by the conductivity of the water in the electrode chambers. The water in these chambers was changed frequently during the first 24 hours of a run; from then on once a day was sufficient.

The water soluble polysaccharides were divided into two major fractions by electrodialysis. One fraction migrated to the positive electrode and was deposited as a gelatinous mass around the positive membrane while the other fraction always remained in suspension during electrodialysis. These fractions were tentatively named alpha and beta respectively. The purification of the alpha and beta fractions of the soluble polysaccharides was readily effected by siphoning the supernatant beta fraction from the chamber. Water was again added to the alpha fraction and electrodialysis was continued until a clear separation had occurred. This process was repeated until the water above the alpha fraction was free of the beta fraction, as evidenced by no precipitate in 60 per cent. alcohol and no coloration with iodine. The purified alpha and beta fractions were precipitated in 70 per cent. alcohol, washed in absolute alcohol, and dried in a vacuum over sulphuric acid at room temperature. Although the beta fraction always remained in suspension during electrodialysis, a portion of it migrated toward the positive electrode. The properties of this portion were identical with those of the non-migrating portion.

Separation and purification of alpha and beta amylose from starch

The starch from air dry mature sweet corn which had been separated from the soluble polysaccharides by centrifuging was purified by washing in 20 and 80 per cent. alcohol. The starch was washed and centrifuged eleven times in 80 per cent. alcohol before it was considered pure.

It is well known that the starch grains must be completely ruptured before a separation of alpha and beta amylose can be made. In order to avoid the possibility of any change in the beta amylose arising from the use of chemical agents, the method of mechanical rupture as reported by Alsberg and Perry (1) and Taylor and Beckmann (22) was adopted. This method gave complete rupture of the starch grains after grinding in a ball mill for one week, as evidenced by the disappearance of the typical black cross on the starch grains when viewed between crossed Nicol prisms.

An 8 per cent. suspension of ground starch in water was subjected to electrodialysis. The alpha amylose migrated to the anode and the clear supernatant beta amylose was siphoned off and precipitated in 75 per cent. alcohol, washed in absolute alcohol, and dried in a vacuum at room temperature. The alpha amylose was repeatedly resuspended in water and electrodialized until free of beta amylose. This required 59 washings. The alpha amylose was then frozen and the frozen mass allowed to thaw as it was filtered in a Buchner funnel. Drying was completed in a vacuum desiccator over sulphuric acid at room temperature.

Physical and chemical methods

Color with iodine.—The iodine coloration of a solution was determined by adding 10 drops of N/100 iodine potassium iodide to 10 ml. of a 1 per cent. solution.

Viscosity.—The relative viscosity was determined with an Ostwald viscosimeter. The measurements were made on 5 ml. of a 1 per cent. solution at 25° C. ± 0.03. All of the determinations were made immediately after preparing the solutions. Each value for relative viscosity is the average of at least two samples and each sample was run through the viscosimeter ten times.

Optical rotation.—A Schmidt and Haensch half-shadow saccharimeter was used to determine the optical rotation. The readings were obtained in Ventzke degrees which were converted to angular rotation by the factor 0.34657. It was necessary to use dilute solutions of the beta soluble polysaccharide preparations because of their opalescence. In determining the optical rotation of samples which had been hydrolyzed, the original weight of the sample was used to calculate the specific rotation. The specific rotations reported for the unhydrolyzed samples are the average of two samples, and five to six readings were made on each sample. The specific rotations for the hydrolyzed materials are from one sample, on which five to six readings were made.

Electrical conductivity.—The apparatus employed for these determinations consisted of the usual equipment for such measurements and was set up as shown by Getman (9). A Leeds and Northrup student's potentiometer was used as a wheatstone bridge. The determinations were made on 1 per cent. solutions at 25° C. ± 0.03. Each conductivity reported is the average of two samples. The conductivity of each sample was determined nine or more times with the fixed resistance at three different points.

Flocculation by salts.—The samples of 10 ml. of 2 per cent. solutions were treated with half saturated ammonium sulphate, basic lead acetate, and 0.1 N iodine potassium iodide.

ASH.—The materials were ashed in platinum crucibles at dull redness in a muffle furnace. The ashings were continued until the crucibles were constant in weight after 30-minute heating intervals.

PHOSPHORUS.—All of the ashings for the phosphorus determinations were made by the wet ashing method following the procedure recommended by ZINZADZE (25). The ashings were performed on an electric sand bath at 165°–170° C., since ROE, IRISH, and BOYD (20) have shown that phosphoric acid is volatized above 200° C. The colorimetric method employed for the beta fractions is that of BELL and DOISY (5) as modified by BRIGGS (6) and improved by ROE, IRISH, and BOYD (20). The procedure for this determination has been reported by COCKEFAIR (7). The standards were prepared from potassium dihydrogen phosphate simultaneously with the unknown solutions. Since ROE, IRISH, and BOYD (20) have shown that the acidity has a marked influence on the color produced, it was necessary to add 1 ml. of concentrated sulphuric acid to the standards in order to make the acidity the same as that of the unknown solutions. The solutions were matched with a Bausch and Lomb Duboscq colorimeter, using 50 mm. cups.

The volumetric method employed for the alpha fractions is essentially that of PEMBERTON (19) as reported by MATHEWS (16). The ammonium phospho-molybdate was washed with water until free of acid, and then dissolved in N/10 sodium hydroxide. This solution was titrated with N/10 sulphuric acid.

FATTY ACIDS BEFORE AND AFTER HYDROLYSIS.—The samples which had been dried at 80° C. under 3 to 4 cm. pressure were extracted in a BAILEY-WALKER (2) extraction apparatus for 24 hours with redistilled anhydrous ethyl ether. The extract was evaporated and dried at 80° under 3 to 4 cm. pressure. Any residue remaining after correcting for the blank determination was considered unbound or extraneous fatty material.

The extracted samples were hydrolyzed according to the method of TAYLOR and NELSON (24) and filtered through fat-free filter paper. The residues were washed free of acid, and after drying the filters and the residues at 50° C., they were extracted with anhydrous ethyl ether. The weight of the ether extract was determined in the manner previously described.

REDUCTION OF FEHLING'S SOLUTION BEFORE AND AFTER HYDROLYSIS.—The reducing power of 50 ml. of 1 per cent. solutions was determined by the Munson and Walker gravimetric procedure (17). The reducing power of samples which had been hydrolyzed for 2.5 hours with 2.5 per cent. sulphuric acid was also determined by this method. The percentage of glucose formed on hydrolysis was calculated by using the factor 0.9, which allows for the increase in weight on hydrolysis.

MELTING POINT OF OSAZONES.—Samples which had been hydrolyzed were neutralized and treated with phenyl-hydrazine hydrochloride and sodium

acetate in a boiling water bath for 1 hour. The resulting osazones were recrystallized and their melting point determined by the capillary tube method. All of the melting points were corrected according to the formula of KOPP (**11**). The melting points reported are the averages of two or three determinations.

NITROGEN.—The nitrogen determinations were made by the usual Gunning method (**17**). Distillation was made into 0.1 N sulphuric acid and the excess acid was titrated with 0.1 N sodium hydroxide using the combination methylene blue-methyl red indicator (**10**).

Results

PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES PREPARED FROM SWEET CORN IN DIFFERENT YEARS

The beta fraction constitutes from 78 to 80 per cent. of the water soluble polysaccharides in sweet corn at the milk stage. The properties of this fraction of the soluble polysaccharides prepared from two different crops of sweet corn in the milk stage were practically identical. Both prepara-

TABLE I

PHYSICAL PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES PREPARED FROM TWO CROPS OF SWEET CORN IN THE MILK STAGE

YEAR	RELATIVE VISCOSITY AT 25° C. ± 0.03	SPECIFIC CONDUCTIVITY AT 25° C. ± 0.03 $\times 10^{-6}$	SPECIFIC ROTATION AT 25° C.	SPECIFIC ROTATION OF HYDROLYZED SAMPLES AT 25° C.
1930	1.0715	9.40	195.8	56.3
1931	1.0750	3.39	195.2	58.7

TABLE II

CHEMICAL PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES PREPARED FROM TWO CROPS OF SWEET CORN IN THE MILK STAGE

YEAR	P_2O_5	CONVERSION TO GLUCOSE ON HYDROLYSIS	MELTING POINT OF OSAZONES FROM HYDROLYZED SAMPLES	TOTAL NITROGEN*
	%	%	° C.	%
1930	0.0079	95.22	208.8	0.05
1931	0.0013	97.96	205.9	0.01

* Single determination.

tions were white amorphous powders which were exceedingly hygroscopic. They were not flocculated by basic lead acetate, half saturated ammonium sulphate, or iodine potassium iodide. Neither of the beta preparations contained any weighable ash or any fatty material. Both preparations were non-reducing and exhibited the same purplish violet iodine color. The results of other physical and chemical measurements on these two preparations are shown in tables I and II. The material prepared in 1930 had a higher conductivity than the material prepared in 1931. This may be due to the fact that the 1930 preparation had more bound phosphorus and also more nitrogen. The nitrogen is probably due to impurities which had not been completely removed from the 1930 preparations.

Comparison of properties of beta fraction of soluble polysaccharides with those of beta amylose prepared from mature sweet corn starch

Preparations of the beta fraction of the soluble polysaccharides from corn in three stages of ripening were compared with one another and with beta amylose from mature sweet corn starch. The solutions of beta fractions of soluble polysaccharides were very opalescent and the iodine color was purplish violet, while the beta amylose solutions were water-clear and gave a pure blue iodine color. There was no ash or bound fatty material in any of the beta fractions or in beta amylose. The materials compared did not reduce Fehling's solution and they were not flocculated by half-saturated ammonium sulphate, basic lead acetate, or iodine potassium iodide. On the addition of a small amount of an electrolyte to the solutions, the beta amylose was flocculated by iodine potassium iodide as reported by

TABLE III

Comparison of physical properties of beta fraction of soluble polysaccharides and beta amylose from sweet corn starch

Material	Relative viscosity at 25° C. ± 0.03	Specific conductivity at 25° C. ± 0.03 $\times 10^{-6}$	Specific rotation at 25° C.	Specific rotation of hydrolyzed samples at 25° C.
Beta fraction of soluble polysaccharides				
Milk stage	1.0750	3.39	195.2	58.7
Dough stage	1.0723	3.35	195.8	55.2
Mature stage	1.0850	6.40	195.8	54.5
Beta amylose	1.1690	15.10	214.0	60.6

BALDWIN (4), but the beta fraction of the soluble polysaccharides was not affected by this treatment. The results of further studies of the physical and chemical properties of these preparations are tabulated in tables III and IV. The viscosities, conductivities, and specific rotations of the beta

TABLE IV

COMPARISON OF CHEMICAL PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES AND BETA AMYLOSE FROM SWEET CORN STARCH

MATERIAL	P_2O_5	CONVERSION TO GLUCOSE ON HYDROLYSIS	MELTING POINT OF OSAZONES FROM HYDROLYZED SAMPLES	TOTAL NITROGEN
	%	%	° C.	%
Beta fraction of soluble polysaccharides				
Milk stage	0.0013	97.96	205.9	0.01*
Dough stage	0.0000	98.37	204.6	0.07*
Mature stage	0.0045	94.50	210.6	0.05*
Beta amylose	0.0042	92.88	209.4	0.006

* Single determination.

fraction of the soluble polysaccharide preparations are less than those of beta amylose. The phosphorus content of the beta fraction from the mature stage and beta amylose is the same, and from the specific rotation of hydrolyzed samples and the melting point of osazones it is evident that the beta fraction of the soluble polysaccharides is converted to glucose on hydrolysis, as is beta amylose.

COMPARISON OF PROPERTIES OF ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES AND ALPHA AMYLOSE

The water soluble polysaccharides from the milk, dough, and mature stages contained approximately 22, 26, and 45 per cent. of the alpha fraction respectively, while the percentage of alpha amylose in the starch was approximately 14 per cent.

The properties of the alpha fraction and alpha amylose which have been compared are shown in tables V and VI. The iodine color of alpha amylose was violet while the iodine color of the alpha fraction was blue. As the corn matures the alpha fraction of the soluble polysaccharides loses in ash, phosphorus, and nitrogen; therefore the compound from mature corn had a higher percentage of conversion to glucose on hydrolysis. The optical rotation of the hydrolyzed samples varied with the stage of ripening of the

TABLE V

COMPARISON OF ASH, PHOSPHORUS, AND NITROGEN CONTENT OF ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES FROM SWEET CORN AND ALPHA AMYLOSE FROM SWEET CORN STARCH

MATERIAL	ASH	P_2O_5	TOTAL NITROGEN
	%	%	%
Alpha fraction of soluble polysaccharides			
Milk stage	0.113	0.674	3.33
Dough stage . .	0.080	0.074	0.94
Mature stage .	0.017	0.025	0.19
Alpha amylose . .	0.800	0.164	0.26

corn from which they were prepared. However, if the weights of hydrolyzed samples were corrected for their percentage of conversion to glucose, the optical rotations approximated that of d-glucose, with the exception of the material from the milk stage, which was lower. The alpha fraction of the soluble polysaccharides from the mature stage and alpha amylose contained a trace of extraneous fatty material, therefore it was either removed or corrected for, in determining the amount of fatty material freed by hydrolysis. The amount of this fatty material increased in the alpha fraction of the soluble polysaccharides as the corn matured. However, there was never as much present in these fractions as there was in alpha amylose. In making the determinations for the fatty material freed by hydrolysis, a flocculent precipitate occurred during the hydrolysis. This was collected

TABLE VI

COMPARISON OF PROPERTIES OF HYDROLYZED ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES FROM SWEET CORN AND HYDROLYZED ALPHA AMYLOSE FROM SWEET CORN STARCH

MATERIAL	CONVERSION TO GLUCOSE ON HYDROLYSIS	SPECIFIC ROTATION OF HYDROLYZED SAMPLES AT 25° C.	MELTING POINT OF OSAZONES FROM HYDROLYZED SAMPLES	FATTY MATERIAL LIBERATED BY HYDROLYSIS
Alpha fraction of soluble polysaccharides	%	%	° C.	%
Milk stage	76.27	36.41	206.2	0.01
Dough stage . .	90.23	51.32	204.0	0.03
Mature stage	91.90	55.25	204.6	0.14
Alpha amylose	92.25	55.93	204.4	0.79

on a filter and washed free of acid before extracting with ether. The alpha fractions of the soluble polysaccharides from the milk and dough stages contained an abundant amount of this flocculent precipitate after hydrolysis, but when this was collected on a filter and washed it was found to be water soluble.

Properties of water soluble polysaccharides obtained from Evergreen sweet corn in milk stage

The water soluble polysaccharides from the Evergreen sweet corn were prepared in the same manner as described for the Hopeland sweet corn. The properties of the beta fractions of the soluble polysaccharides were very similar to those of the Hopeland fractions, with the exception that the Evergreen fractions had a higher conductivity.

The properties of the alpha fraction of the soluble polysaccharides from the Evergreen corn were similar to those of the Hopeland alpha fraction in the milk stage, with the exception that the Evergreen material had more ash and more fatty material freed by hydrolysis.

Discussion

The data on the beta fraction of the soluble polysaccharides indicate that it is possible to obtain from sweet corn a preparation which will have fairly consistent properties from year to year. The change in properties of this fraction as the corn matures may be due to an increase in the chain length of the compound. Therefore an attempt was made to determine the molecular weights of the different preparations by the cryoscopic method, but it was not possible by this method to obtain consistent results. In comparing the properties of the beta fraction of the soluble polysaccharides with those of beta amylose, it was evident that the physical properties of these two compounds are different. The former was readily soluble in cold water and gave an opalescent solution while the latter was difficultly soluble in cold water and gave a water-clear solution. The beta amylose had a greater viscosity, conductivity, and optical rotation than the beta fraction of the soluble polysaccharides. The chemical data indicate that these two compounds differ only in their physical properties. The specific rotation of the beta amylose was higher than that reported by other investigators (**4**, **24**) working with beta amylose prepared from other kinds of starch.

The solubility of the alpha fraction of the soluble polysaccharides and alpha amylose was similar. Both compounds were readily soluble before electrodialysis; however, after repeated electrodialysis it was exceedingly difficult to get either compound into a stable suspension. Owing to this fact such physical measurements as viscosity, conductivity, and optical rotation would be of doubtful value.

The properties of the two fractions of the water soluble polysaccharides which have been investigated are not similar to any of the dextrins resulting from starch hydrolysis, as reported by SAMEC (**21**). Some dextrins resulting from direct hydrolysis of starch have been reported by BAKER (**3**) and by LINTNER and DÜLL (**15**) as having specific rotations which were very similar to that of the beta fraction of the soluble polysaccharides; however, the other properties reported by these investigators for their dextrin preparations were quite different from the properties of the alpha or beta fractions of the soluble polysaccharides. LINK (**14**) isolated and purified a dextrin from the radicle and plumule of the young corn plant. This dextrin was not similar to either fraction of the soluble polysaccharides found in sweet corn endosperm.

LAMPE and MEYERS (**13**) have studied the development of the endosperm of sweet corn microchemically, and report that globules first form and that carbohydrate grains which give characteristic starch reactions may or may not form within these globules. They advance the theory that these globules contain the water soluble polysaccharides. This idea was later given more strength by LAMPE (**12**). These investigators also report that they found no evidence of a reversal or a hydrolysis after the carbohydrate grains were once formed.

In the light of these recorded observations and the fact that the present investigation shows that the water soluble polysaccharides contain two fractions which in some respects are similar to sweet corn starch components, it is possible that the water soluble polysaccharides present in sweet corn are the units for the formation of starch grains.

Summary

1. The water soluble polysaccharides present in sweet corn endosperm were isolated and divided into two major fractions by electrodialysis. One fraction migrated to the positive electrode and was deposited as a gelatinous mass around the positive membrane while the other fraction always remained in suspension during electrodialysis. These fractions have been tentatively named alpha and beta, respectively.

2. The physical and chemical properties of these fractions of the soluble polysaccharides from corn in the milk, dough, and mature stages were compared with each other and with alpha and beta amylose prepared from sweet corn starch.

3. As the corn matured many of the properties of the alpha and beta fractions of the soluble polysaccharides approached those of alpha and beta amylose of starch but they departed widely from those that have been described for dextrins.

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SEASONAL MARCH OF CARBOHYDRATES IN *ELYMUS AMBIGUUS* AND *MUHLENBERGIA GRACILIS*, AND THEIR REACTION UNDER MODERATE GRAZING USE

EDWARD C. MCCARTY

(WITH THREE FIGURES)

Introduction

In a study of the western wheat grass, *Agropyron smithii*, the seasonal march of the total concentration of the sugar and starch fractions was found to vary inversely with the rate of growth of the herbage. The storage of these carbohydrates occurred during the declining phase of herbage growth, near the close of the annual growth cycle (2). A subsequent study of the seasonal march of the carbohydrates of the needle grass, *Stipa pulcra*, showed inverse proportionality between the rate of herbage growth and the total concentration of the sugar and starch fractions, in both the herbage and the stem bases and roots of the plant. Carbohydrate storage was also delayed until the closing phase of the annual growth cycle (5). In each of these grasses, one clipping treatment, applied at the onset of the interval of carbohydrate storage, resulted in a maximum diminution in these food materials in the stem bases and roots of the plant, at the close of the growing season.

In view of the economic importance of these facts in the management of range lands, it is obviously desirable to extend these studies to other grass species. The object of the present study therefore, was to determine: (1) the normal progress of the carbohydrate foods in the herbage and in the subterranean organs of the plant; (2) the period of food accumulation; and (3) the effect of a single harvest made at various stages of the annual growth upon the accumulation of carbohydrate food in the perennial organs of the plant.

The experimental plots were located at Ruxton, on the eastern slope of Pike's Peak, in Colorado. The immediate area was a grass covered hillside situated at the upper limit of the transition life zone, and with a gentle slope to the southeast. The soil was relatively deep on the slope, showing some rock outcrop and a loose admixture of gravel. The grasses studied were wild rye, *Elymus ambiguus* Vasey and Scribn., and a dropseed, *Muhlenbergia gracilis* Trin. These grasses were predominant on the area although there were other grasses and a sparse population of low herbs present.

At weekly intervals height growth data were secured by measuring 50 shoots, selected at random over the area. The dry weight increment was determined by drying the shoots in a ventilated oven at 65° C. (table I). Upon plotting these data, cumulative curves, sigmoid in character, were

TABLE I

HEIGHT, GROWTH, AND DRY WEIGHT OF *ELYMUS AMBIGUUS*; 1930

DATE	AVERAGE HEIGHT GROWTH	PERCENTAGE	AVERAGE DRY WEIGHT	PERCENTAGE
	cm.	%	*gm.*	%
June 24	30.8	52.5	0.130	11.8
July 3	36.0	61.4	0.218	19.8
July 10	36.5	62.4	0.221	20.1
July 18	38.7	63.0	0.225	20.4
July 30	45.7	78.0	0.319	29.0
Aug. 7	54.3	92.6	0.440	40.0
Aug. 15	57.1	97.6	0.649	59.0
Aug. 27	57.8	98.8	0.703	63.0
Sept. 4	58.6	100.0		
Sept. 18			1.100	100.0

obtained. Rate curves were constructed by drawing lines tangent to the cumulative curves at convenient points, and computing the tangent of the angle made by the convergence of these lines upon the x-axis (1), (table II; figs. 1, 2).

The herbage samples as prepared for analysis consisted of the entire shoots, clipped 1 inch from the soil surface. The basal organs, consisting of stem bases, rhizomes, and roots, were combined for the purpose of analysis. Upon collection and preparation, these respective samples were placed in a ventilated oven and dried for a period of 24 hours at 65° C.

TABLE II

RATE OF GROWTH OF *ELYMUS AMBIGUUS*

HEIGHT GROWTH		DRY WEIGHT	
DATE	$\frac{Y}{X}$	DATE	$\frac{Y}{X}$
June 14	1.10	June 14	0.30
June 24	1.50	June 24	0.52
July 4	0.32	July 4	0.27
July 14	0.08	July 14	0.04
July 24	0.10	July 24	0.62
Aug. 3	2.04	Aug. 3	1.30
Aug. 13	0.52	Aug. 13	1.20
Aug. 23	0.10	Aug. 23	0.28
Sept. 2	0.00	Sept. 2	2.40
		Sept. 12	0.60
		Sept. 18	0.00

After grinding, the samples were dried for an additional 24 hours at the same temperature. The sugars were then extracted with alcohol. The residue remaining after the extraction was ground to a fine powder in a ball mill, and the starch fraction obtained by salivary digestion. The suspension was filtered through quantitative paper in a Büchner funnel. From this residue the hemicellulose was obtained by hydrolysis, using 2.5 per cent. concentrated hydrochloric acid. All solutions were cleared with basic lead acetate. The reductions were carried out as described in a previous paper (**3**).

Observations

Growth of the plants

The annual growth of a perennial range grass shows two general periods, characterized respectively by the development of the leafy shoots and by the lengthening of the flower stalks. The growth of the vegetative shoots is at first accelerated until the maximum rate is reached, after which the rate of growth declines. During this interval the inflorescence is differentiated from the apical meristem, thus initiating the second period of growth. The growth of the flower stalks is likewise accelerated, and as the rate declines, the fruit ripens and the plant reaches maturity.

In the wild rye grass the growth of the flower stalks progressed very slowly at first, and the heads appeared generally over the plots June 20. Following the change in acceleration of the rate of growth of the flower stalks, the dry weight of the plant became greatly accelerated (fig. 1). Depression in the rate of increase in dry weight was apparently correlated with flowering and the production of fruit, a condition found also in *Avena fatua* (**3**). As in this latter grass, the dry weight lagged behind the increase in height growth. Although the height growth had ceased on September 4, further increase in dry weight was indicated in the collection of September 18.

The growth of adventitious roots was observed July 30. This growth was completed in perhaps two weeks, after which the growth of secondary shoots began. The occurrence of new root and shoot growth during the declining phase of the annual growth cycle shows agreement with *Stipa pulcra* (**5**). Alternation in growth was found also in the study of *Avena fatua* (**3**). Parker and Sampson report root growth to be more active during the "autostatic" phase in *Stipa pulcra* and *Bromus hordeaceus* (**4**).

The annual growth of the plant was presumed to have been completed by September 18. At this time the fruit was in process of dissemination and the plants had assumed a characteristically mature aspect. There had also been a hard frost prior to this date.

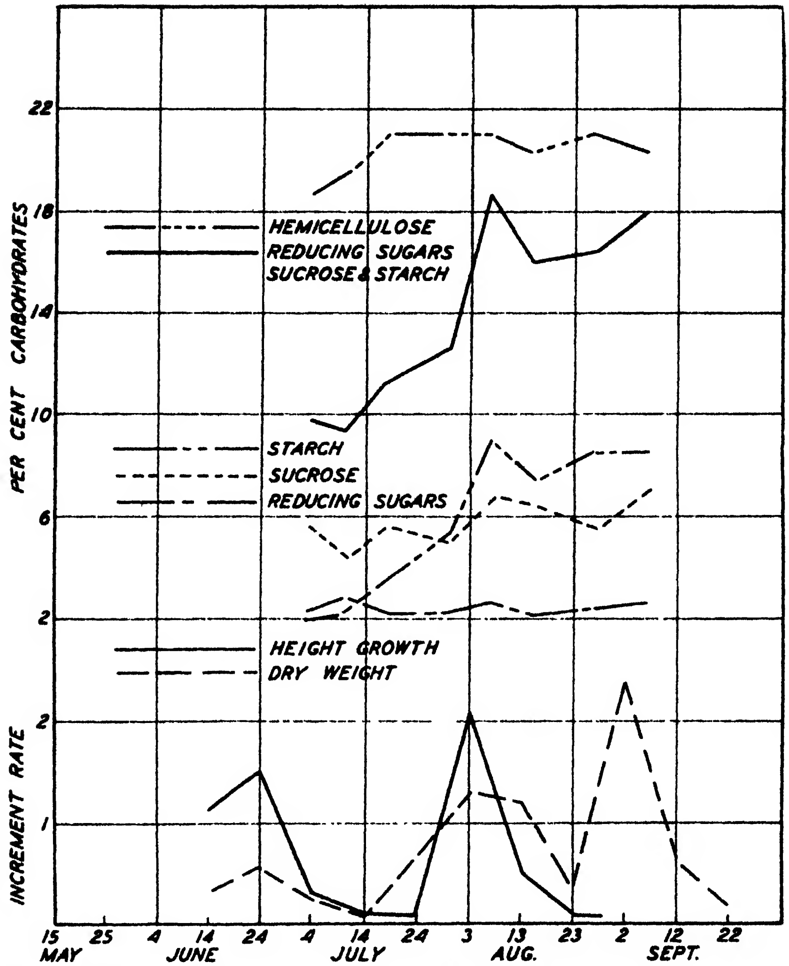

FIG. 1. *Elymus ambiguus*: upper, behavior of sugar, starch, and hemicellulose fractions in herbage of untreated plants (table IV); lower, rate of growth of the leafy shoots in height and their increase in dry weight (table II).

The principal growth features of *Muhlenbergia gracilis* were similar to those of *Elymus ambiguus*, although there were some differences in the time of appearance of the flower stalks and in the maturity of the fruit. Root growth, however, practically coincided in both grasses.

GROWTH OF THE PLANTS UNDER USE

Moderate use was approximated by clipping, one harvest being made of each plot successively as shown in table III. In all groups clipped during the period of vegetative activity some renewed growth occurred. The

renewed growth following clipping was not disturbed by any further treatment, and on September 6 the stem bases and roots were collected and

TABLE III

AVERAGE HEIGHT GROWTH OF CLIPPED PLOTS OF *ELYMUS AMBIGUUS*, 1930

PLOT	DATE OF HARVEST	AVERAGE HEIGHT BEFORE CLIPPING	AVERAGE HEIGHT SEPT. 6	TOTAL AVERAGE HEIGHT GROWTH
		cm.	*cm.*	*cm.*
1	June 24	30.8	31.6	62.4
2	July 3	36.0	24.8	60.8
3	July 10	36.5	19.8	56.3
4	July 18	38.7	17.2	55.9
5	July 30	45.7	8.0	53.7
6	Aug. 7	54.3	Appreciable	
7	Aug. 27	57.8	Appreciable	

prepared for analysis. Growth was renewed immediately following the harvest of plot 1, on June 24, but was somewhat delayed in the plot harvested on August 7. Whereas renewed growth was vigorous and immediate in plot 1, there was a progressive decline in the amount of growth made following the clipping of each successive plot.

If the total average height growth for the season is considered to be the sum of the average height growth at the time of clipping and the average height growth at the final harvest of each plot, there is an apparent increase in this factor when comparison is made with the untreated plants (tables III, I). A single clipping may both delay and diminish tillering, however; also, if flower stalks are produced, their diameters and ears of grain may be decidedly smaller than in the untreated plants (3). The yield following clipping is therefore lower than in the untreated plants.

MARCH OF THE CARBOHYDRATES

HERBAGE.—The concentration trends of the carbohydrates in the plant may be shown advantageously by means of the curve representing the sum of the reducing sugars, sucrose, and starch fractions (figs. 1, 2). In general the trend is from low concentrations in the early part of the growing season to high concentrations, the maximum occurring during the declining phase of growth at the close of the annual growth cycle. The increase in these carbohydrates between June 24 and the close of the season was more than 8 per cent., based upon the dry weight of the plant. The greatest change in concentration was found in the starch fraction, which increased rapidly following the turning point in growth rate of the vegetative shoots. The fluctuations in the sucrose level were smaller than those of the starch

fraction, but in both the trend was from low concentrations early in the growth cycle to high concentration at the close of the growing season. Only very slight changes occurred in the level of the reducing sugars throughout the period of observation. The variations in the acid hydrolyzable fraction, hemicellulose, were less than those of the starch fraction. A decline in the hemicellulose concentration, however, is shown at the time of flowering and the development of the fruit (table IV, fig. 1).

TABLE IV

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN HERBAGE OF *ELYMUS AMBIGUUS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR, 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGARS, SUCROSE, AND STARCH	HEMICELLULOSE
		%	%	%	%	%	%
447	July 3	2.30	5.57	7.87	1.88	9.75	18.67
449	July 10	2.77	4.49	7.26	2.14	9.40	19.47
451	July 18	2.30	5.57	7.87	3.48	11.35	21.07
453	July 30	2.30	4.96	7.26	5.32	12.58	21.07
455	Aug. 7	2.69	6.81	9.50	9.08	18.58	21.07
457	Aug. 15	2.14	6.40	8.54	7.40	15.94	20.27
459	Aug. 27	2.38	5.49	7.87	8.48	16.35	21.07
463	Sept. 6	2.61	6.89	9.50	8.48	17.98	20.27

TABLE V

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN STEM BASES, RHIZOMES, AND ROOTS OF *ELYMUS AMBIGUUS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR. CALCULATIONS MADE UPON AN ASH FREE BASIS; 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGAR, SUCROSE, AND STARCH	HEMICELLULOSE	ASH
		%	%	%	%	%	%	%
446	June 24	2.06	3.35	5.41	2.68	8.09	25.68	4.68
448	July 3	1.61	3.93	5.54	4.95	10.49	25.68	4.69
450	July 10	1.64	3.77	5.41	5.87	11.28	27.38	4.72
452	July 18	0.99	3.12	4.11	5.77	9.88	23.60	4.43
456	Aug. 7	1.08	2.36	3.44	6.52	9.96	22.76	4.22
458	Aug. 15	1.20	2.98	4.18	5.98	10.16	22.76	4.29
460	Aug. 27	1.13	2.45	3.58	6.92	10.50	23.76	4.52
464	Sept. 6	1.17	3.29	4.46	8.26	12.72	22.80	4.55
482	Sept. 18	1.17	4.63	5.80	8.26	14.06	22.80	4.59

STEM BASES, RHIZOMES, AND ROOTS.—The reducing sugars, sucrose, and starch in the stem bases, rhizomes, and roots showed trends similar to those in the herbage (table V, fig. 2). Low concentrations prevailed during the earlier phases of herbage growth, and maximum levels occurred with the decline in the growth rate at the close of the growing season. As in the herbage, the variations in the starch were greater than those obtaining in

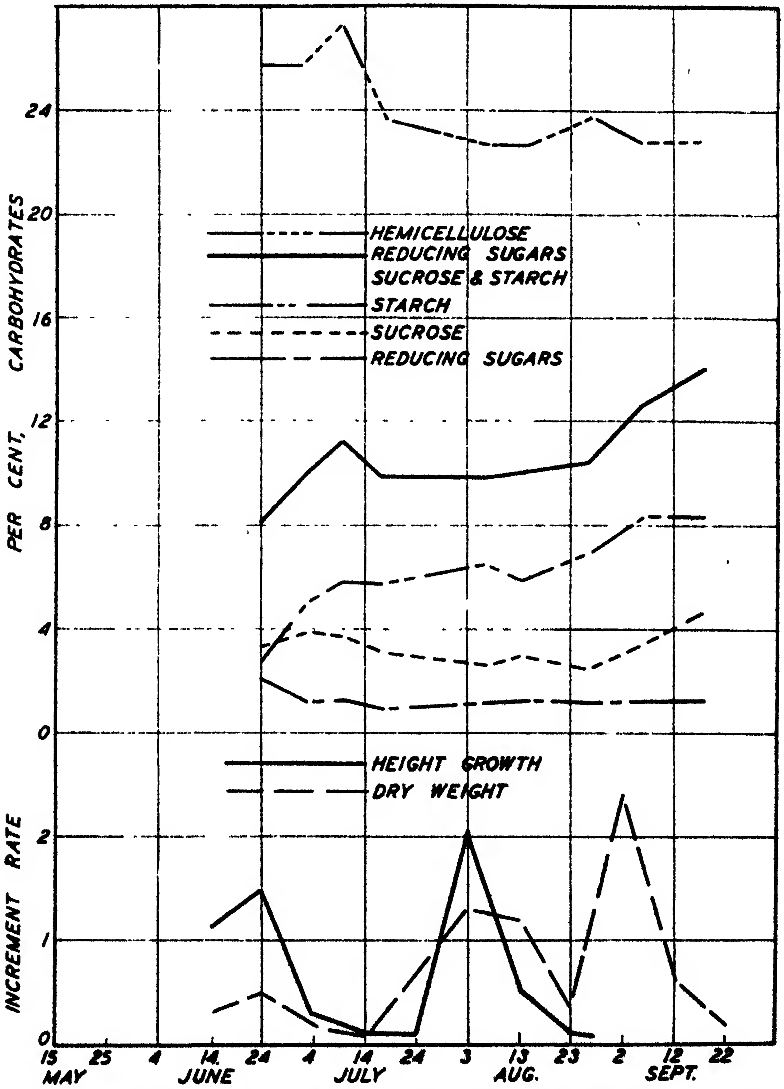

FIG. 2. *Elymus ambiguus*: upper, behavior of sugar and starch fractions in stem bases, rhizomes, and roots of untreated plants (table V); lower, rate of growth of the leafy shoots in height, and their increase in dry weight (table II).

the sucrose, and in both fractions the concentration trends were from low values on June 24 to high values at the end of the annual growth cycle. The reducing sugars were maintained at a more or less constant level throughout the period of observation. The concentration of the acid hydrolyzable hemicellulose declined during the reproductive period, showing agreement with the behavior of this fraction in the herbage.

MARCH OF THE CARBOHYDRATES IN *MUHLENBERGIA GRACILIS*.—Essentially the same trends are shown in the march of the carbohydrates in the stem bases and roots of *M. gracilis* as in the rye grass. The sugars exceeded

TABLE VI

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN STEM BASES AND ROOTS OF *MUHLENBERGIA GRACILIS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR; 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGARS, SUCROSE, AND STARCH	HEMICELLULOSE
		%	%	%	%	%	%
421	June 24	0.78	2.83	3.61	1.78	5.39	36.32
425	July 10	1.15	3.84	4.99	1.88	6.87	36.32
427	July 18	0.77	3.48	4.25	1.88	6.13	36.32
429	July 30	0.83	2.59	3.42	3.12	6.54	36.32
431	Aug. 7	0.86	2.56	3.42	2.66	6.08	33.92
433	Aug. 15	0.77	2.33	3.10	2.00	5.10	34.32
439	Sept. 9	0.86	4.13	4.99	1.36	6.35	34.32
441	Oct. 18	0.89	4.64	5.53	3.36	8.89	33.92

TABLE VII

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN STEM BASES AND ROOTS OF TREATED PLOTS OF *ELYMUS AMBIGUUS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR. CALCULATIONS MADE UPON AN ASH FREE BASIS; 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGAR, SUCROSE, AND STARCH	HEMICELLULOSE	ASH
		%	%	%	%	%	%	%
466	June 24	1.97	3.69	5.66	7.08	12.74	26.33	6.79
468	July 3	1.39	3.88	5.27	5.44	10.71	24.96	9.47
470	July 10	1.19	2.72	3.91	5.32	9.23	25.46	7.46
472	July 18	0.99	2.81	3.80	6.30	10.10	25.41	7.40
474	July 30	0.95	2.62	3.57	6.76	10.33	25.65	7.78
476	Aug. 7	0.92	2.00	2.92	7.18	10.10	25.74	8.03
480	Aug. 27	1.04	2.39	3.43	5.70	9.13	23.47	6.64

the starch fraction in amount. The greater part of the sugar fraction was found to be sucrose, however, and this sugar is probably the more important storage product. The accumulated foods are laid down during the closing phase of the annual growth cycle, showing agreement with the wild rye grass (table VI).

Carbohydrate concentration in clipped plots.—As previously stated, only one clipping was made of each plot, and the stem bases and roots were removed September 6. The relative values of the several carbohydrate fractions were in agreement with those in the untreated plants. As in the untreated plants, the starch values were higher than either sucrose or the reducing sugars. Based upon the amount of accumulated carbohydrate foods, the series is progressive, the largest amount being contained in the group harvested June 24 (table VII, fig. 3).

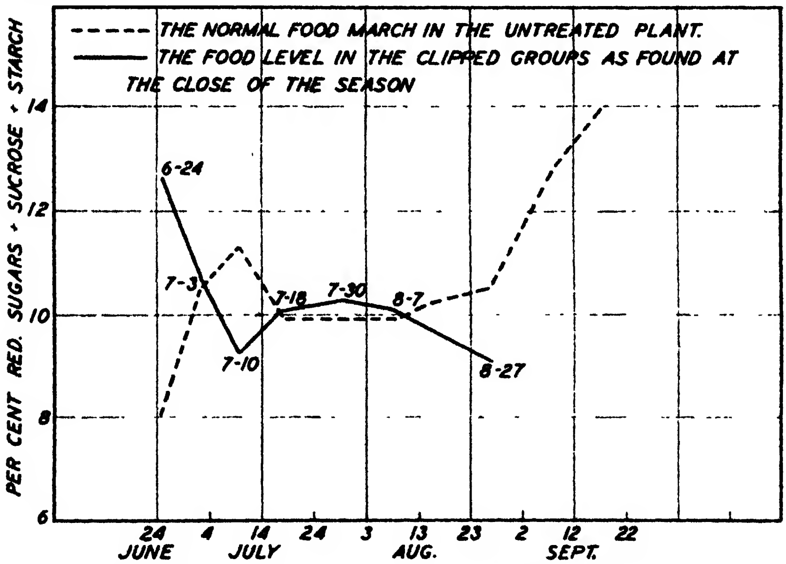

Fig. 3. *Elymus ambiguus*: total accumulation of sugar and starch fractions in the clipped groups, as found at close of annual growth cycle (table VII). The dates show when the single clipping treatment was applied in each group. Following these respective dates no further clipping treatments were applied, and the stem bases and roots of each group were collected on September 6. The curve representing the normal behavior of the total of the sugar and starch fractions is repeated for convenience.

Discussion

The annual growth cycle of *Elymus* is characterized by series of developmental steps, each occurring in sequence, each involving meristem activity, and each drawing upon the available carbohydrate foods of the plant. In the order of their occurrence, these steps comprise the development of the leafy

shoots, the initiation of the flowers, the elongation of the flower stalks, the growth of new adventitious roots, and the development and maturity of the fruit.

Although the photosynthetic area of the plant increases rapidly during the early period of herbage growth, utilization of the carbohydrate foods in respiration and in tissue building is apparently very great in proportion to the food making capacity of the herbage. Moreover, tillering occurs during this early period and the rate of growth is progressively accelerated. Carbohydrate utilization therefore continues at equal pace with the increase in leaf area and the synthesis of carbohydrate foods, and in consequence low concentrations prevail in the herbage during the early period of vegetative growth. The increase in the carbohydrate concentration coincident with the change in growth acceleration appears to be related to a decline in the rate at which these foods are utilized in the several growth processes, as well as to the increased photosynthetic area of the plant.

The march of the reducing sugars, sucrose, and starch in the stem bases and roots is inversely correlated with the rate of growth of the herbage. Previous studies have shown that the accumulations of these carbohydrates are greatly depleted within a relatively short time after the resumption of growth, following a rest period (5). Low values continued in the basal structures during the period of accelerated shoot growth, but increases in the several fractions followed the turning point in the growth rate. The inverse correlation prevailing between growth and the carbohydrate level in the stem bases and roots is evidence of the utilization of these materials at a rate quite in excess of their production, and hence production becomes a limiting factor in the several growth processes. Root growth is seen to alternate with growth of the herbage, occurring during the "autostatic" phase of growth. The carbohydrate concentration in the stem bases and roots remained constant during root growth, suggesting that meristem activity in the roots, as well as in the herbage, tends to monopolize the available food supply of the plant (3). With the conclusion of these growth phenomena, food accumulation was effected, the upturn of the concentration beginning on August 27 (table V, fig. 2).

As shown by the average height of the plants (table III), the most complete recovery was made in the group clipped on June 24. The amount of renewed growth made by each succeeding group decreased progressively, being only appreciable in the group clipped on August 27. Since the increment values in the untreated plants were greatest during the months of June, July, and August (table I), the external environmental factors may be presumed to have been favorable during these months. The amount of growth made by the several groups was proportional to the number of days remaining in the growing season following the clipping of each group (table

III). Food accumulation in the treated plants, as in the controls, occurs during the declining phase of growth, and both treated and untreated plants tend to reach this "autostatic" growth phase at the same time (2). Food accumulation in *Elymus ambiguus* agrees with that in *Agropyron smithii*, therefore, and is correlated with the amount of herbage present at the onset of the period of accumulation (2).

Renewed growth following clipping or grazing must be made at the expense of the accumulated carbohydrate foods in the stem bases and roots of the plants. Such growth is invariably followed by a diminution in the seasonal level of these foods in the storage organs (5, 3). This decline in the seasonal carbohydrate level tends to be proportional to the rate of growth at the time the clipping treatment is applied (3). Furthermore such diminution represents a loss of potential energy and imparts some injury to the plants. The contrast between the accumulated foods in the treated and in the untreated plants is shown graphically in figure 3.

There is reason to know that such diminution in carbohydrate foods as is found in the several groups of treated plants does not necessarily result in death of the plant. The amount of these foods found in the group clipped on August 27 is sufficient to promote fair growth during a subsequent season. Such diminutions serve to prevent maximum yields, however, and to lessen the number of fruit stalks produced during the subsequent season's growth. Again the decline in food level resulting from use tends to be cumulative when treatment is repeated from year to year.

Although the longer period of growth between the clipping of the first group on June 24 and the final upturn of the food level in the stem bases and roots yielded the most favorable results in the process of accumulation of carbohydrate foods in the stem bases and roots of the plants, this fact cannot be construed to favor early use of range lands. Further carbohydrate studies have shown specific injuries imparted by clipping or grazing during the first few weeks of seasonal growth. On the other hand the total annual growth of herbage is not essential to the production and accumulation of maximum amounts of carbohydrate foods. Moreover the results of the treatments applied to the several groups show the value of the late seasonal growth in the process of carbohydrate accumulation in the stem bases and roots of the plants.

Summary

1. The grasses *Elymus ambiguus* and *Muhlenbergia gracilis* were studied in their native habitat.

2. The seasonal march of the carbohydrates, as shown by the sum total of the sugars and starch, was found to be in inverse ratio to the rate of growth of the herbage.

3. Secondary growth of roots and shoots occurred during the closing phase of the growth cycle, and the carbohydrate concentration in the stem bases and roots remained practically constant during this growth activity.

4. The accumulation of carbohydrate foods was completed some time between September 7 and 18. Much of this food was apparently elaborated by the secondary herbage growth.

5. In the treated plants the highest and the lowest concentrations of the accumulated carbohydrate foods were found respectively in the group clipped June 24 and August 27, the latter being approximately at seed maturity.

6. In the clipped groups, the amount of herbage growth made subsequent to treatment and the concentration of the accumulated carbohydrates were roughly proportional to the number of days between the date of the clipping treatment and the end of the annual growth cycle.

The field work incident to the preparation of this paper was made possible by Dr. FREDERIC E. CLEMENTS, who also placed the facilities of the Alpine Laboratory at the disposal of the writer. The final collections of samples were made by Mr. LEONARD JOHNSON. Mrs. E. C. McCARTY also assisted, both in the field and in the laboratory.

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GROWTH AND SEASONAL CHANGES IN COMPOSITION OF OAK LEAVES

ARTHUR W. SAMPSON AND RUDOLF SAMISCH

(WITH FOUR FIGURES)

Introduction

Study of growth and of seasonal changes in composition of leaves of certain western species of oak was undertaken as part of an investigation to determine forage values and certain management phases of the oak associations. An attempt was made to record seasonal changes in the major constituents of the leaves. The results are expressed (1) on a relative basis, namely, that of dry weight and of unit leaf area, respectively, and (2) on an absolute basis of leaf unit. The paper discusses the merits of the two bases for a study of this character.

Methods

Leaf samples were collected from *Quercus gambelii* Nutt. and *Q. kelloggii* Newb., both deciduous. *Q. gambelii*, a scrub form, occupies foothills and low mountains from southwestern Wyoming through Mexico and Arizona (**8**), whereas *Q. kelloggii* (**6**) is essentially a California species, occurring between elevations of 500 to 8000 feet throughout the length of the state (**3**).

Leaf samples of *Q. gambelii* were obtained from the same clumps during the entire growing seasons of 1930 and 1931, respectively.[1] In addition to this, a series of leaf samples was collected in 1931 from another oak clump located about 1 mile from the first clump. The analytical data pertaining to the samples gathered on the same date from these clumps agreed closely. The values for the growth rate of *Q. gambelii* consist of the averages for the corresponding samples from the two selected clumps (table I). Since no measurements of leaf area were made during the season of 1930, the data in the following tables pertain to samples gathered in 1931 only. These data are believed to be representative, however, since the analytical results obtained from leaf samples collected in 1930 show the same trend as those procured a year later.

Leaf samples of *Q. gambelii* were taken at approximately monthly intervals. The first samples were collected in June, when the leafage had unfolded sufficiently to afford browse for grazing animals. Flowering had been completed and acorns were beginning to form. By September the fruits attained full size, and many were being cast before reaching maturity.

[1] The samples of this species were collected through the courtesy of the Director of the Intermountain Forest and Range Experiment Station, near Ephraim, in central Utah.

At that time autumn color was still absent in the leaves. By the end of October all the leaves and the acorns had abscissed. The last sample, obtained on October 24, had to be collected from the ground.

Leaf samples of *Q. kelloggii* were collected during the growing season of 1931, near St. Helena in Napa County, California. The first sample was obtained in July, when the leaves had reached about half of their maximum season's growth. In September the leaves were still green. On December 1, when the last sample was collected, the leaves had turned partially brown, only a portion along the midrib still being green.

The average dry weight and the average area per leaf were determined as follows: leaves in lots of 300 were counted and weighed. Fifty leaves were blue-printed and their area measured with a planimeter. The two sets of leaf samples obtained from the two clumps of *Q. gambelii* checked closely. The figures as reported in table I for the leaves of this oak represent averages of 600 and 100 leaves, respectively.

Rate of growth

The rate of increase in area and the increase of the leaf in dry weight are shown in table I.

TABLE I

GROWTH RATE OF *QUERCUS GAMBELII* AND *Q. KELLOGGII*

	Time of harvest				
	June 21	July 3	Aug. 6	Sept. 8	Oct. 24
	Q. gambelii				
Average area per leaf in square inches ..	1.09	2.84	3.43	3.92	4.52
Average dry weight per leaf in mg.	69	166	207	235	205
	June 11	July 19	Sept. 13	Dec. 1	
	Q. kelloggii				
Average area per leaf in square inches ..	5.78	8.09	10.3	10.3	
Average dry weight per leaf in mg.	432	646	658	649	

After a rapid growth rate during the first month under observation, the rate of expansion of leaves of *Q. gambelii* decreased measurably. The increase of leaf area, expressed in percentage of leaf area of each of the preceding samples, was 160 per cent. for the period between June 11 and July 2, 21 per cent. between July 3 and August 6, and 14 and 15 per cent.,

TABLE II

CONSTITUENTS OF DECIDUOUS OAK LEAVES IN GRAMS PER 1000 LEAVES
SAMPLES COLLECTED 1931

	Quercus gambelii					*Quercus kelloggii*			
	June 11	July 3	Aug. 6	Sept. 8	Oct. 24	June 21	July 19	Sept. 13	Dec. 1
Dry weight	69.4	166	207.5	233	205	432	646	658	649
Water	151.8	313.3	204.5	235	207	379	....	....	549
Ash	3.7	7.7	9.5	10.5	9.9	19.0	34.6	35.2	41.4
Crude protein	16.9	30.5	30.8	31.5	9.9	51.8	73.5	58.3	44.2
Crude fiber	10.2	40.4	44.0	43.4	48.6	76.1	121.2	129.0	127.2
Ether extract	0.8	4.5	5.5	8.4	8.9	10.6	18.7	24.8	26.8
N-free extract, by difference	37.8	82.9	117.7	139.2	127.7	274.5	398.0	410.7	409.4
SiO_2	0.23	0.51	0.95	1.36	1.57	2.07	4.97	6.91	12.28
CaO	0.40	1.76	2.59	3.02	3.50	5.48	8.26	8.22	12.01
P_2O_5	0.85	1.32	1.35	1.74	1.31	2.76	5.23	4.80	4.86
K_2O	1.36	3.05	3.26	3.34	2.60	5.09	9.18	9.87	6.36
Na_2O	0.13	....	....	....	0.31	0.48	....	0.94	0.96
Cl	0.01	....	....	....	0.05	0.07	....	0.13	0.17

respectively, for September and October. In *Q. kelloggii* a corresponding increase of 40 per cent. was observed during the first month, compared with a total increase of 27 per cent. during the following two months. There was no further increase in area after September 13. Apparently the stage of growth in *Q. kelloggii* was somewhat more advanced at the time the experiment was started than was that of *Q. gambelii.*

A similar trend was also observed in the increase of dry weight per leaf. The decrease in weight of the last collected sample of *Q. gambelii* (October 24) may be attributed either to leaching or to translocation of nutrients from the leaves to the branches.

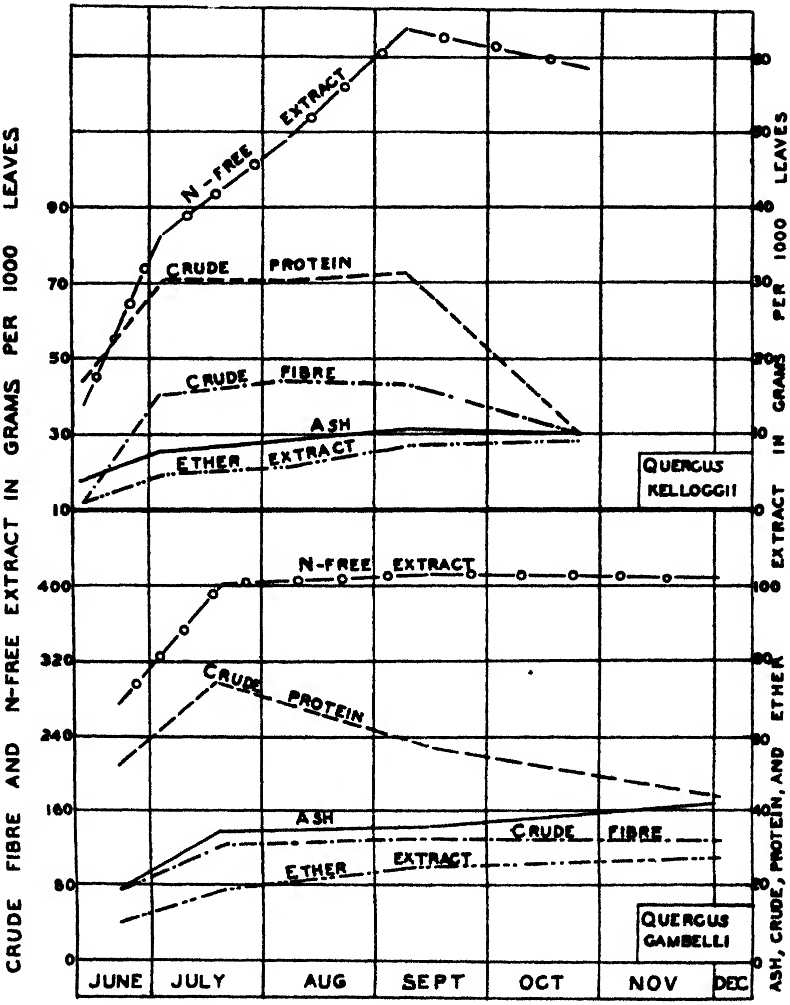

FIG. 1. Major constituents of oak leaves, expressed in grams per 1000 leaves.

On the basis of the growth data presented it was deemed advisable to distinguish three stages of leaf development: (1) the period of rapid growth, (2) the period of maturation, and (3) the period of senescence.

Chemical changes

The analytical methods used in this study will be published in detail elsewhere; with slight modification, they were those adopted by the Association of Official Agricultural Chemists.

In table II and figures 1 and 2, the analytical results are presented on the basis of grams per 1000 leaves. In table III and in figures 3 and 4, the

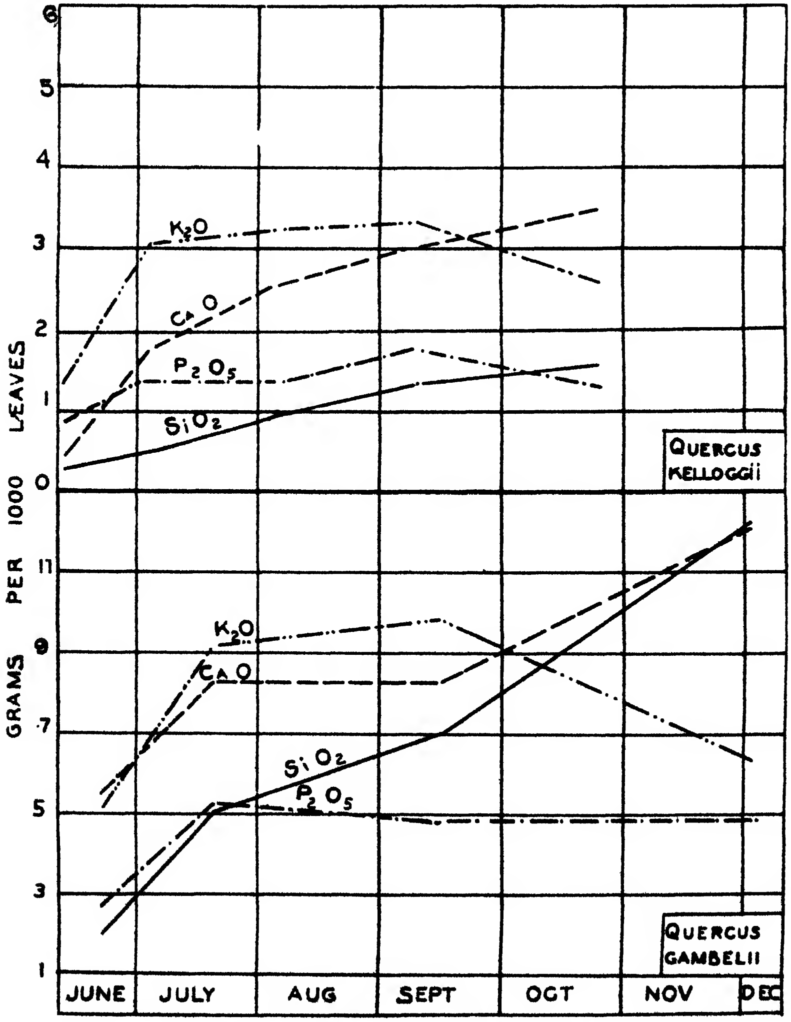

FIG. 2. Mineral constituents of oak leaves, expressed in grams per 1000 leaves.

data are expressed on a leaf area basis, namely, in grams per 100 square inches of leaf surface.

In the following discussion changes in constituents are shown for the three stages of leaf development. For convenience, the data presented for

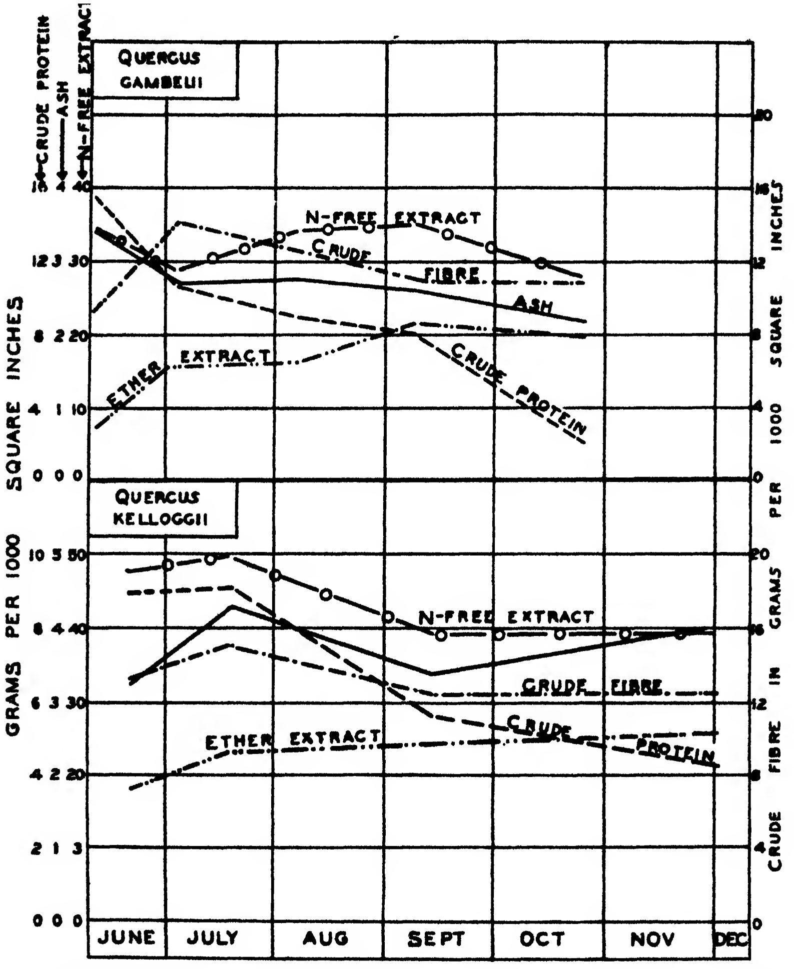

FIG. 3. Major constituents of oak leaves, expressed in grams per 1000 square inches.

Q. kelloggii under the heading which follows are placed in parenthesis immediately after the corresponding ones for *Q. gambelii*.

1. Period of rapid growth

This period includes the first month under observation. The basic data are given in table II and their relative trends are shown in figure 1.

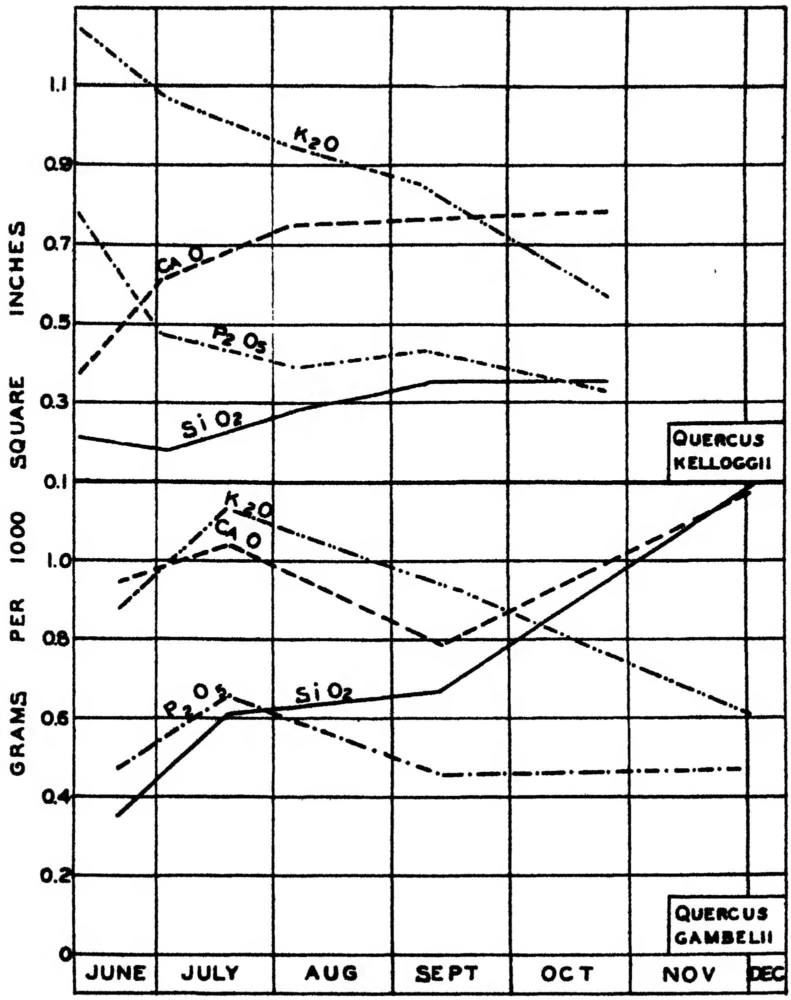

FIG. 4. Mineral constituents of oak leaves, expressed in grams per 1000 square inches of leaf surface.

The period of very active growth is characterized by a rapid accumulation of salts and carbohydrates. From table II it has been calculated that, within approximately one month after the first collection of leaf material, there was an increase per leaf of 108 (82.6) per cent. in ash; 80 (42) per cent. in protein; 298 (59) per cent. in fiber; 463 (76) per cent. in ether extract; and 119 (59) per cent. in nitrogen-free extract. Throughout the early growth period *Q. kelloggii* showed a slower rate of accumulation of the constituents just compared. This behavior may be explained by the fact that the first sample of *Q. kelloggii* was collected at a more advanced

growth stage than was the corresponding sample of *Q. gambelii*, as already pointed out.

Toward the end of the rapid growth period, as on July 3 and 19, respectively, there had accumulated in the leaves 73 (83) per cent. of the total of ash taken up during the entire season; 51 (70) per cent. of the ether extract; and 60 (97) per cent. of the nitrogen-free extract. The more advanced leaf development of *Q. kelloggii* is again reflected in a correspondingly more pronounced accumulation of the various constituents.

It is of interest to note that the entire amount of protein, 97 (100) per cent., and most of the crude fiber, 83 (94) per cent., was already present in the leaf at this early season; whereas relatively little accumulation of ether extract was in evidence. This very rapid accumulation of protein and of some ash constituents during the early period of growth might be attributed, in part at least, to the fact that during this stage of growth nitrogenous compounds, as well as some other constituents, are largely obtained from the storage organs of the plant. Thus BAUER (1), working with one-year-old oak seedlings, has shown that about 60 per cent. of the nitrogen intake of the leaves during early growth is derived from the storage organs. During this period of active growth, leaf area increased at a very rapid rate, as previously pointed out. In *Q. kelloggii* the growth rate was virtually equal to that of the accumulation of nitrogen in the leaves. But in *Q. gambelii* leaf expansion proceeded at a more rapid rate than did that of protein accumulation, thus resulting in a decrease of crude protein per leaf area. (Compare crude protein for June 11 and July 3, table III and fig. 3.)

CRUDE FIBER.—The behavior of crude fiber, which can be regarded as a measure of ligno-cellulose, resembles that of protein inasmuch as most of it is accumulated during the stage of active growth; but the rate of accumulation is much more rapid, and even exceeds that of leaf expansion. This is reflected by increase of crude fiber per unit area of 52 per cent. in *Q. gambelii* and of 13 per cent. in *Q. kelloggii*.

Morphologically, ligno-cellulose is a function of cell division and cell growth. An additional factor which has a bearing on the amount of ligno-cellulose in the leaves is the development and increase of the fibrovascular system and the lignification of the pedicel of the leaf.

NITROGEN-FREE EXTRACT.—This consists largely of sugars, hemicelluloses, and pectins. The expansion of photosynthetic surface was accompanied by the accumulation of these substances in the leaves. The increase of these constituents in *Q. gambelii* (Utah) did not quite keep pace with the rate of leaf expansion, whereas in *Q. kelloggii* (California) its accumulation exceeded the rate of growth.

ASH.—The ash constituents (table II, fig. 2), similar to the organic compounds, accumulated rapidly during the period of early growth. During

TABLE III

CONSTITUENTS OF DECIDUOUS OAK LEAVES IN GRAMS PER 1000 SQUARE INCHES

SAMPLES COLLECTED 1931

	Quercus gambelii					*Quercus kelloggii*			
	June 11	July 3	Aug. 6	Sept. 8	Oct. 24	June 21	July 19	Sept. 13	Dec. 1
Average area per leaf	1.09	2.84	3.43	3.92	4.52	5.78	8.09	10.35	10.27
Dry weight per 1000 square inches	64.3	58.4	60.4	59.4	45.4	74.7	80.0	63.6	63.2
Water	139.3	110.3	59.95	59.95	4.58	65.4	...	...	53.4
Ash	3.09	2.71	2.77	2.62	2.19	3.28	4.28	3.40	4.03
Crude protein	15.50	10.74	8.97	8.03	2.19	8.98	9.10	5.64	4.30
Crude fiber	9.36	14.21	2.81	11.08	10.87	13.18	14.91	12.48	12.40
Ether extract	0.73	1.58	1.60	2.14	1.97	1.84	2.32	2.39	2.61
N-free extract, by difference	34.62	29.16	34.25	35.53	28.27	47.42	49.39	39.69	39.86
SiO_2	0.21	0.18	0.28	0.35	0.35	0.36	0.61	0.67	1.19
CaO	0.37	0.62	0.75	0.77	0.78	0.95	1.04	0.79	1.17
P_2O_5	0.78	0.97	0.39	0.44	0.29	0.48	0.65	0.46	0.47
K_2O	1.25	1.07	0.95	0.85	0.58	0.88	1.13	0.95	0.62
Na_2O	0.12	...	...	...	0.07	0.083	...	0.091	0.093
Cl	0.009	...	...	...	0.012	0.0012	...	0.0012	0.0016

this period calcium increased 340 (51) per cent., potassium 124 (80) per cent., silica 122 (140) per cent., and phosphorus 55 (89) per cent. These great differences in rate of accumulation of the various ash constituents are seen more clearly when plotted on the basis of unit area (fig. 4).

While changes per leaf represent absolute values of accumulation or loss, a comparison of changes on a unit area basis involves two variables, namely, the rate of leaf expansion and the rate of intake of constituents. In *Q. gambelii* (fig. 2) there was a rapid accumulation of all elements in the individual leaf when compared on a leaf basis, whereas figure 4 shows that phosphorus, potassium, and even silica decreased in *Q. gambelii* when plotted on a per unit area. In other words, the rate of intake of these constituents was smaller than the rate of leaf expansion. On the other hand, where there was a slower growth rate, such as in *Q. kelloggii*, all elements were on the increase during this early period of growth, regardless of whether the data were calculated on the leaf area bases or on the basis of a single leaf.

Similar trends were obtained when the results were reported as percentage of dry weight, for rate of leaf expansion and rate of increase in weight happened to run parallel at this early stage of development. Data are frequently reported in the literature as percentage of weight, seldom on the basis of unit area. It is often difficult to gain a picture of the absolute accumulation of or loss of materials unless a given organ or plant is taken as a basis of reference. The importance of this point in interpretation of the analytical results has been strongly emphasized by WEHMER (9).

2. PERIOD OF MATURATION

This period covered the months of July and August. It was characterized by a substantially reduced rate of growth and by a slow increase in all constituents. It will be noted (fig. 1) that in *Q. kelloggii* resorption of nitrogen, and possibly of phosphorus (fig. 3), had already taken place.

In the leaves of *Q. gambelii* the smallest increase was in protein. Prior to this period most cells had ceased to divide. Enlargement of the leaves was proceeding at a somewhat more rapid rate than was the increase in those constituents. They showed, therefore, an apparent decrease when calculated on a unit area basis. The decrease of crude fiber per unit area was due to the relative increase of the area of the leaf proper, as compared with the veins and stem of the leaf, which are known to be comparatively rich in ligno-cellulose.

During this period (samples collected August 6, September 8 and 13) ether-soluble substances increased at a fairly rapid rate whereas crude fiber and potassium remained practically stationary in both species. Accumulation of nitrogen-free extract almost ceased in *Q. kelloggii* whereas it continued at a moderate rate in the leaves of *Q. gambelii*. Silica increased at

a constant rate in both species. This was also the case with calcium in the leaves of *Q. gambelii*. It seems peculiar, however, that in *Q. kelloggii* there was no intake of calcium for a period of two months.

Although silica is frequently regarded as an external contamination, this seems to be the case only to a limited extent with the material used in this investigation, which was carefully wiped before grinding. If the presence of silica were due merely to dust deposit, then it would hardly have showed fluctuation in the rate of deposition per unit area, as exhibited by the present material.

During the period of maturation certain constituents showed an apparent loss in the leaf, when compared on a leaf area basis with samples collected earlier in the season, despite the fact that in absolute amounts, *i.e.*, per leaf unit, these constituents had actually accumulated in the leaves or had at least remained stationary. Such is the case for crude protein in *Q. gambelii*, for nitrogen-free extract in *Q. kelloggii*, and for crude fiber in both species.

3. Period of senescence

This phase is characterized by a resorption of a number of constituents from the leaves of deciduous trees into branches, trunk, and roots. The early literature on the subject was discussed by Wehmer (9) and by Swart (7). More recently the translocation of nitrogen compounds from the leaves to other organs of trees has been reported by Combes (2) and by Lincoln (4).

In the present work, one leaf sample from *Q. kelloggii* and from *Q. gambelii*, respectively, was obtained during the period of senescence (tables II, III). The sample of *Q. gambelii*, collected October 24, consisting of dead leaves collected from the ground, showed a loss in protein, phosphorus, and potassium. This behavior might have been the result of leaching rather than of translocation of these constituents from leaves to twigs and branches.

On the other hand, the leaves of the sample of *Q. kelloggii*, collected December 1, also showed a loss in nitrogen, phosphorus, and potassium. These leaf samples were alive and had not abscissed. It is highly probable, therefore, that the losses in constituents previously enumerated were due to their resorption from the leaves and presumably not to leaching. In *Q. kelloggii* the amount of phosphorus in the leaves did not change between September 13 and December 1; but a significant portion of the phosphorus and the protein was translocated from the leaves between July 19 and September 13. Resorption of phosphorus and of nitrogen evidently started in this species at an earlier period than did that of potassium. On the other hand, silica and calcium continued to accumulate in the leaf whereas crude

fiber and ether extract accumulated slightly or remained at a stationary level.

Summary

1. Leaves of *Quercus gambelii* (Utah) and of *Q. kelloggii* (California) were collected at more or less regular intervals during the growing season. They were analyzed after their area and their dry weight had been measured.

2. Protein was found to have accumulated in the leaf at a very early stage of growth. Although no more nitrogen was taken up during the remainder of the season, leaf expansion continued, resulting in a reduction in protein content per unit area. In the latter part of the growth period there was an actual resorption of protein from the leaf into the storage organs.

3. Most of the crude fiber, like that of protein, was accumulated early in the growth period, after which it increased only very slowly. It decreased per unit area during the latter part of the season because of the more rapid leaf expansion during this period.

4. Ether-soluble substances accumulated continuously throughout the growing season.

5. The nitrogen-free extract increased very rapidly in the California species studied, and remained almost constant after the initial rise (July); whereas it continued to accumulate gradually throughout the season in the Utah species, at least as late as September.

6. Calcium and silica increased at a rapid rate throughout the growing period.

7. Potassium showed a rapid rise in spring, followed by a somewhat declining increase during the summer. This slow rate of increase during the summer, as compared with leaf expansion, resulted in a drop of potassium per unit area. There was a pronounced resorption of potassium in the fall.

8. Phosphorus showed an early peak in the California leaf samples, whereas this peak did not occur before September in the Utah species.

9. This study emphasizes that in order to follow seasonal changes in the *absolute* amounts of chemical constituents occurring in specific plant organs, or in entire plants, the data should correspondingly be expressed on the basis of a specific organ, such as the leaf, or the plant as a whole.

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MODIFICATION OF LEAF STRUCTURE BY X-RAYS

YAKICHI NOGUCHI

(WITH SIX FIGURES)

Introduction

The effect of x-rays upon seeds and seedlings very often causes abnormalities of form and changes in the internal structure of the plant. The cells of a treated radicle show elongation, great vacuolation or entire lack of protoplasm, and absence of nuclei from many cells (**1, 6**). Strong irradiation sometimes causes tumor-like tissues at the root tip, which give a bulbous and swollen appearance and which contain multinucleated giant cells (**4, 7**). Fasciations of stems, leaves, and flowers in the sunflower and bushy forms of young tomato plants occur after treatment (**1, 3**). Abnormality of leaves is a very common injury by x-rays: the leaves become asymmetric, distorted, pocked; light green areas intermingle with ordinary green as if the leaves were suffering from a mosaic disease (**1, 3, 10**).

Very few studies have been made on the structure of these abnormal leaves. The writer found that one investigator has reported the interference with the normal development of palisade cells and spongy parenchyma which takes place in such leaves (**3**).

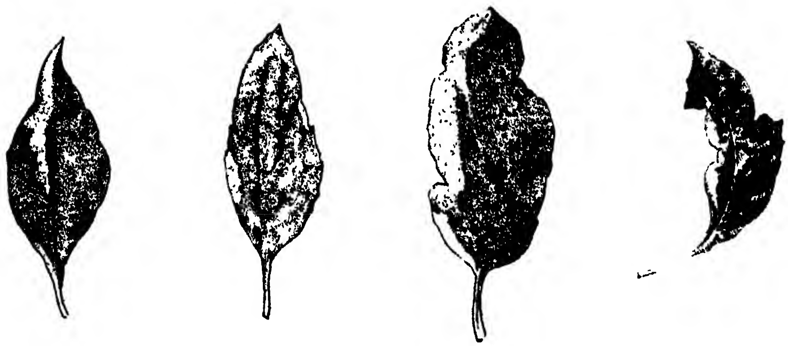
Material and methods

Since Russian sunflower, *Helianthus annuus*, is very sensitive to x-rays and abnormality of vegetative parts can easily be produced by irradiation, this plant was selected for these experiments. Seeds were placed for about 24 hours in a moist chamber on a layer of cellucotton saturated with distilled water, and kept at a temperature of 25° C. Some investigators (**2, 5, 8, 9**) found that the sensitivity of seeds to x-rays is influenced by the amount of water they contain at the time of treatment; therefore it is necessary to measure the water content of the material. The average water content of air-dried seeds of *Helianthus* is 3.75 per cent. After keeping them in moisture for 24 hours this increases to 38.83 per cent. and the seeds show incipient germination. At this stage, after removing the pericarps, as uniform seeds as possible were selected for treatment and controls. The irradiation used in these experiments was made with an x-ray machine in the following set-up: 100 K.V. 5 ma. current, no screen, 30 cm. focal distance. Immediately after treatment both irradiated seeds and controls were planted in pots and kept under environmental conditions as much alike as possible, in the greenhouses of the University of Chicago.

Results

General aspects of leaf abnormality

The most common deformities presented by leaves after treatment are asymmetry of blade and distortion caused by small pockets. Light or dark green (occasionally both) areas intermingle with ordinary green, and, very rarely, even colorless portions appear along the margin of the leaf (fig. 1, *a–c*). With strong irradiations the first two or three leaves show a peculiar aspect, as if they were suffering from a mosaic disease (fig. 1, *d*).



(a) (b) (c) (d)

Fig. 1. Variations of color produced in leaves by x-ray irradiation: *a*, light green areas in ordinary green leaf; *b*, dark green scattered streaks; *c*, colorless area along the margin; *d*, mosaic diseased appearance.

An experiment has been performed with the time element as the only variant in the production of abnormalities in leaves. The seeds were divided into five series of ten seeds each, and irradiated for 5, 10, 15, and 20 minutes respectively, the remaining lot being unradiated, as control. Observations were made after ten weeks, when the plants were nearly mature. Table I records the abnormalities observed.

Histology of normal leaf

Cross sections of young leaves about 1.5 cm. long, from normal plants which were used as controls, were examined to determine the normal development of the tissues in the early stages. One or two layers of long but uniform palisade cells lie under the epidermis, and the space between the palisade tissue and the epidermis of the under surface of the leaf is filled with small cubic spongy parenchyma. All the cells in young tissue appear light green with compactly arranged chloroplasts. As the leaves grow the palisade cells become longer and the spongy parenchyma cells become angular in shape. The green elipsoidal chloroplasts are found

TABLE I

Leaf abnormalities appearing after irradiation of x-rays: M, heavy mosaic diseased appearance; m, light mosaic diseased appearance; L, light green areas intermingled with normal green; l, small light green areas intermingled; D, dark green areas interspersed with normal green; w, colorless areas apparent; N, normal

No. leaves / No. plants	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Series I (20 minutes' treatment)																		
1	M	M	the bud arrests growth															
2	M	M	Lw	L	l	l	ml	Ml	l									
3	M	M	L	D	l	l												
4	m	m	L	LD	l	lw	lw											
5	M	M	L	L	L	L	l	l										
6	M	M	L	L	L	L	L	L										
All leaves asymmetric and distorted; four plants died from heavy injuries before the emergence of leaves																		
Series II (15 minutes' treatment)																		
1	M	M	m	l	l	a side bud appears												
2	M	M	L	l	l	N	N	l	N									
3	m	m	L	L	the bud arrests growth													
4	M	L	L / N	L / N	L	two buds develop												
5	m	m	L	L	l	N	l	l	l									
6	M	M	m	m	L	L	l	l	l									
7	m	L	L	N	N	N	N	N	N	N	N	N	N	N				
All leaves asymmetric; three plants died in seedling stage																		
Series III (10 minutes' treatment)																		
1	L	L	L	l	l	l	N	N	N	N								
2	m	m	N	N	N	N	N	l	N									
3	L	L	l	l	N	N	N	N	N	N	N	N	N					
4	L	L	l	l	l	N	N	N	N	N	N	N	N					
5	m	m	L	l	l	N	N	N	N	N	N	N						
6	M	M	m	m	L	L	l	l	l	N	N	N	N	N				
7	m	m	D	d	N	N	N	N	N	N	N	N	N					
8	m	m	m	L	L	l	l	N	N	N								
9	M	M	L	L	l	l	l	N	N									
All leaves slightly asymmetric and pocked; one plant injured by insects is excluded from the table																		
Series IV (5 minutes' treatment)																		
1	m	m	l	l	N	N	N	N	N	N	N	N	N					
2	m	m	N	N	N	N	N	N	N	N	N	N						
3	m	m	l	N	N	N	N	N	N	N	N	N	N	N	N			
4	L	L	N	N	N	N	N	N	N	N	N	N	N					
5	m	m	m	m	N	N	N	N	N	N	N							
6	m	m	N	N	N	N	N	N	N	N	N	N	N					
7	m	m	L	L	L	l	l	N	N	N	N	N	N					
8	m	m	l	l	N	N	N	N	N	N	N	N	N	N	N	N	N	N
All leaves symmetric; two plants injured by insects																		

gathered closely along the inside cell wall. In the mature leaf (4 cm. in length) the arrangement of cells is very regular.

Histology of abnormal leaf

Colorless part.—The appearance of colorless parts is rare; it seems to be a severe injury since it was induced only in a series of strong irradiation. The structural aspect of the leaf is just like that of an ordinary variegated leaf. There is no disturbance of tissue in the green part, although the color is somewhat lighter, probably owing to the defective chlorophyll-absorbing power of the chloroplasts. On the boundary between colorless and green tissues, in most cases the abnormal development of both palisade and spongy cells is found in the colorless area. The palisade cells are, in some instances, unusually slender, frequently curved, smaller, and more spherical. Discrepancies often occur in place of the regularity of the normal palisade arrangement. No differentiation between palisade and parenchyma occurs in the center of the colorless section when x-ray dosage is severe. A few chloroplasts without chlorophyll are found in cells of colorless tissue.

Light green part.—Two kinds of structures are found in the light green section of the leaf. Sometimes the color is caused by the small quantity of chloroplasts in the cells; in such cases no change of structure occurs. Usually, however, the development of tissues, especially palisade, is abnormal, accompanied by a lack of chloroplasts. In the normal leaf palisade cells stand side by side very compactly, but in the abnormal leaf, owing to the irregular arrangement, small or large spaces appear between palisade cells (fig. 2). When the injury is considerably severe, the entire

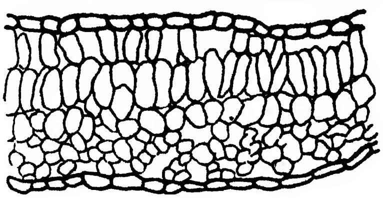


Fig. 2. Abnormal structure of light green parts. Small or large spaces appear between palisade cells owing to their irregular arrangement.

leaf appears light green with small irregular streaks of normal or dark green scattered over the surface, and the texture of the leaf becomes exceedingly coarse. Significant interference of development between palisade and spongy parenchyma is indicated in the light green areas of such leaves (fig. 3). The chloroplasts in the abnormal cells are more spherical and smaller than in the normal; they also seem to lack the normal power of absorption of chlorophyll.

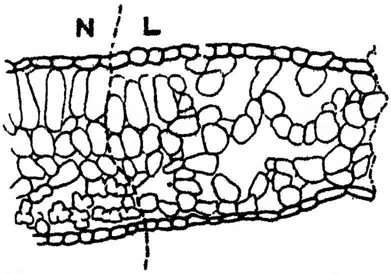


FIG. 3. Strong interference with development of palisade and parenchyma in the severely injured light green area: N, normal; L, light green parts.

DARK GREEN PART.—Dark green irregular streaks appear scattered over the leaf surface. This deep color is due to the extremely strong chlorophyll-absorptive power of the chloroplasts.

MOSAIC-DISEASED APPEARANCE.—The most interesting leaves were those showing the mosaic-diseased appearance. Distinct disturbance of development occurred in all tissues, and when the cross section of the leaf was observed under the microscope, unusual structures were frequently found. The arrangement of epidermal cells was more or less wavy or zigzag. Within a single microscopic field of vision the cells ranged from dark to normal to light green in color (fig. 4). Occasionally one or two layers

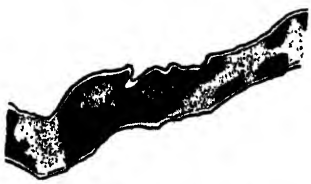


FIG. 4. Cross section of mosaic leaf showing dark, normal, and light green areas.

of colorless cells were found directly beneath the epidermis. From these observations it is readily concluded that the structure of the abnormal leaf is very complicated, and even in the least disfigured leaf several structural abnormalities will be found.

The general aspects of structure of the light and normal green areas are the same as those already mentioned (fig. 5, left, middle). The structure of the dark green areas, however, is entirely different from that of the dark green area described above.

The distinction between palisade and parenchyma tissues in most cases no longer exists. The deformed chloroplasts, clustered in groups of approximately six to eight, fill the cells and are a more bluish green. Frequently, moreover, the chlorophyll diffuses through the cell sap; thus the leaf appears even darker. The walls of the parenchyma cells are thin and less distinct. In the lower layer of the parenchyma coagulation of the

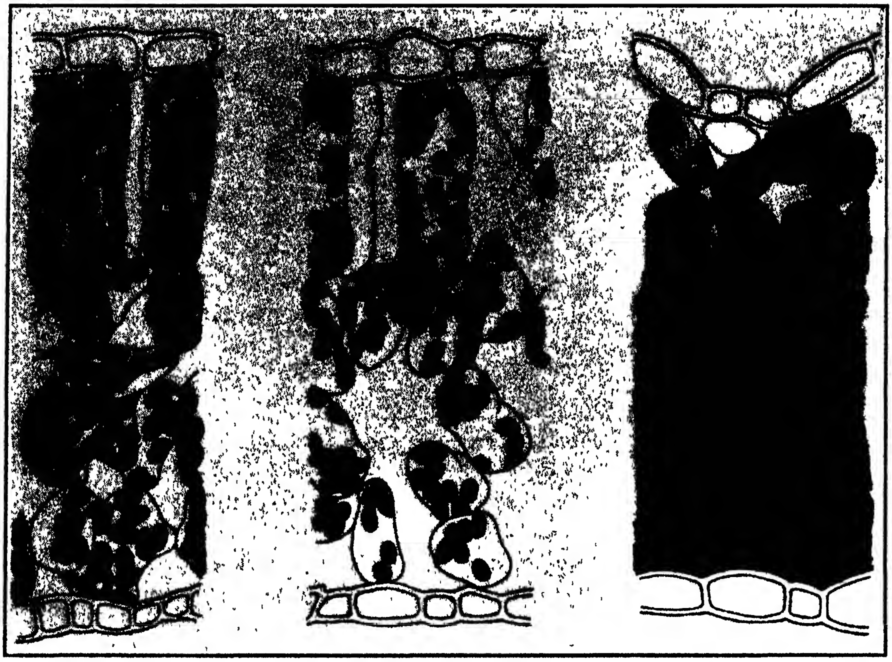


FIG. 5. Internal structures of mosaic leaf: left, normal; middle, light green; right, dark green portions.

chloroplasts occurs and the cell walls are even less distinct (fig. 5, right). Pockets are caused by the gradual or sudden interruption of a layer of palisade cells.

After the longest periods of irradiation a very peculiar structure is found in the leaf with the mosaic appearance. All of the cells become

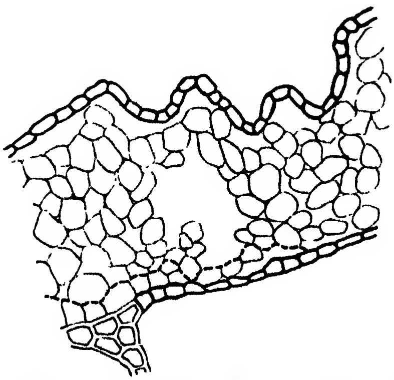


FIG. 6. Strong abnormality of structure after severe irradiation by x-rays. All the cells become globose and similar and are accompanied by one layer of colorless cells (under the broken line).

globular and similar and contain only a very small number of chloroplasts or none at all (fig. 6). The abnormality of structure can be seen even in the early stages of a treated leaf.

Effect of minimum dose of x-rays

It has been reported by several investigators that comparatively strong x-rays induce leaf abnormality, but apparently the minimum limit has not been determined. When seeds which had been imbibing water for 24 hours were treated longer than five minutes, the abnormalities appeared on the leaves. Table II summarizes the results of three experiments, including a brief description of abnormalities occurring after treatment.

TABLE II

Relation of abnormalities to length of irradiation period. Each experiment was repeated three times and twelve seeds were used in each series

No. weeks after treatment	Time of treatment			
	5 minutes	10 minutes	15 minutes	20 minutes
3	None	First two leaves show mosaic appearance	First two or three leaves like those of mosaic plants*	Severity of injury kills half the plants; abnormality severe even in early stages
4	Very slight abnormalities at tip of leaf	Abnormality comparatively severe and growth of plant irregular	Abnormalities very severe	Abnormalities very severe
5	”	”	”	Growth of plant arrested by injury
6	”	”	”	Very severely injured; plants die later

* One plant had leaves which showed only slight injuries.

The results given in table II show that the minimum limit is less than a 5-minute irradiation period. A further experiment was performed to determine the shortest period of treatment that would produce injury. Five series of plants which were treated 1–5 minutes showed very slight abnormalities at the tips of the leaves in the series treated for four minutes,

four weeks after treatment; the same abnormality was recognized in a series treated for three minutes five weeks after treatment.

Localization of injuries induced by x-rays

Whether the effect of x-rays upon seedlings is localized or not is one of the interesting problems connected with leaf abnormality. Three sets of experiments were performed using seeds which had been imbibing water for 1, 2, and 3 days respectively. No essential difference could be seen in treatment with seedlings of different stages, however, and only a summary of the results will be given.

In a series of experiments, germinated seeds were treated in one set with a metallic screen (3 mm. copper plate) protecting the tops and in another with the screen protecting the roots. The third series was treated without the screen. The fourth, untreated, served as a control. A remarkable difference was observed in the time taken by the seedlings to emerge from the soil. Of the seeds treated for 20 minutes, after they had been planted a week, the control showed 100 per cent. emergence, the top-screened 41 per cent., the root-screened 50 per cent., but none of the unscreened seeds emerged. Generally the root-screened seedlings emerged somewhat more rapidly than the top-screened, while the unscreened were extraordinarily retarded in emergence. Irradiation of the bud retarded growth, so that the control and top-screened seedlings showed almost the same rate of growth; but the elongation of the stem in the other two series was much retarded. The average height of the plants in the latter case is about one-half or one-third of the height of the control plants.

No abnormality appears on the leaf when the top of the plant is protected by the screen; root-screening alone has no effect in preventing appearance of top abnormality, even if the degree of injury is somewhat less than that of the unscreened seeds. The shorter the irradiation period the less the difference of leaf abnormality in the four series (control, top-screened, root-screened, and unscreened).

A complete localization of injuries by x-rays is found in the histological investigation. The incomplete development of vascular bundles in the stem, the abnormality of root cells, and the appearance of dark brown color in the hypocotyl region from the treatment by x-rays, as Johnson (1) reported, were also found and their localization observed in these experiments.

Summary

1. The seed of the sunflower is sensitive to x-rays and often shows abnormal development of vegetative parts on germination after treatment. Abnormality of the leaves is the most common symptom, however, and is constantly induced by a strong irradiation. The leaves become asym-

metrical and crumpled in appearance and develop more or less sinuous contours; they also frequently show irregular coloring of blades. Usually light, dark green, or colorless areas intermingle with ordinary green; even such a peculiar aspect as apparent symptoms of mosaic disease occurs when the injury is severe. Of course, the longer the period of irradiation of the seeds the more marked is the abnormality of the leaf.

2. The internal structure of the colorless part is like that of the ordinary variegated leaf. Cells of palisade tissue are small and slender, frequently curved, and their arrangement is disturbed by the occasional absence of cells.

3. Two kinds of structure are found in light green areas. One is normal as the light color is caused only by the small number of chloroplasts in each cell; the other shows disorder of palisade tissue with much space between cells and an accompanying lack of chloroplasts, which probably have some deficiency in their chlorophyll-absorbing power. Noticeable interference between palisade cells and spongy parenchyma occurs when the entire leaf shows light green color with small streaks of darker green.

4. In the leaves in which dark green tissue is intermingled with normal green tissue no peculiar variations can be found. The deeper color is due only to the greater accumulation of chlorophyll in the chloroplasts.

5. The structural aspect of the leaf with mosaic appearance is more complicated. The four areas, colorless, light green, dark, and normal green, intermingle throughout the leaf structure. The structure, however, is just like that of the preceding color sections except the dark green areas. Strong disturbance takes place in all tissues: the distinction between palisade and parenchyma cells disappears and the chloroplasts aggregate in groups, and sometimes coagulate, giving rise to a bluish green color. Moreover, chlorophyll diffusing out into the cell sap produces a deeper green color.

6. The minimum time limit for inducing leaf abnormality by x-rays is 3 or 4 minutes' irradiation under the following conditions of radiation: 100 peak K.V., 5 ma. current, no screen, 30 cm. focal distance.

7. X-ray injuries seem to be localized and confined to directly treated regions, and to definitely localized cellular tracts. The apparent injury and structural modifications are both localized.

These experiments were performed at the botanical laboratory of the University of Chicago. The writer expresses his thanks to Professor C. A. Shull for his kindness in suggesting this problem and for his advice in carrying out the experiments.

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OBSERVATIONS ON THE CRACKING OF CHERRIES[1]

Z. I. Kertesz and B. R. Nebel

(WITH ONE FIGURE)

Introduction

The cracking of the fruit is an important problem in most sweet cherry districts. Precipitation during harvest often occasions to growers heavy losses from cracking.

There is little information on this subject in the literature. Hartman and Bullis (4) find that in sweet cherries moisture absorption occurs through the roots or through the epidermis of the fruit itself following increased osmotic pressure caused by increases of sugars and soluble solids. Verner and Blodgett (9) find that cracking in sweet cherries is due to absorption of water through the skin of the fruit. This is influenced directly by the osmotic concentration of the fruit juice, the skin of the fruit, and skin permeability. Gardner, Bradford, and Hooker (3) suggest that elasticity of the skin may be a controlling factor in cracking of fruits.

In the present paper results are reported of cracking tests on cherries of different varieties, together with anatomical and physiological observations. The problem is yet far from an actual solution. It is believed, however, that a few points established and some physico-chemical relations suggested warrant preliminary publication, especially since these studies cannot be continued in the near future.

Morphological considerations

Five cherries from each of seven varieties and from one seedling (Bing, Lambert, Napoleon, Giant, Schmidt, Yellow Spanish, English Morello, and an F_1 seedling, Lambert × Schmidt) were selected and fixed in a weak solution of acetic acid, formalin, and alcohol. Longitudinal strips were cut in such a way that microtome sections could be made equatorially in the neighborhood of the ventral suture. Sections were stained with hematein. Measurements of the epidermal cells were made with an adjustable Spencer ocular micrometer. The subepidermal cell layers were measured by drawing them with a camera lucida upon uniform stock paper, numbering each cell, cutting out approximately 20 cells at a time, and calculating the area from the weight. The cubic content was calculated by multiplying the square root of the square value with the latter, this being admittedly a rough approximation only.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 62, December 13, 1934.

An arbitrary distinction is made in that cells from the single surface layer only, covered by the cuticle, were measured as epidermis. All secondary layers of epidermal cells from the outermost one inward to the parenchyma of the flesh proper are called subepidermis. This definition is necessary because, as pointed out by STEVENS (8), it is often impossible to distinguish sharply between epidermis and subepidermis. The size of the epidermal cells in the seven varieties and one seedling used in this study was measured, but no correlation could be found between size of cells and extent of cracking. For instance, English Morello and Bing showed relatively small epidermal cells while Napoleon and Yellow Spanish showed relatively large epidermal cells, yet English Morello and Yellow Spanish resist cracking while Bing and Napoleon crack severely. Furthermore, the sour cherry Montmorency, which does not crack, has epidermal cells similar in size to those of Bing, Lambert, Giant, and Schmidt.

While no correlation could be found between the size of epidermal cells and cracking, an unexplained positive correlation seems to exist between the thickness of the inner wall of the epidermis and the extent of cracking. The results are shown in table I.

TABLE I

RELATIVE THICKNESS OF INNER WALL OF EPIDERMIS OF 8 CHERRY VARIETIES

VARIETY	RELATIVE THICKNESS
Lambert	100
Bing	95
Lambert x Schmidt	95
Napoleon	95
Schmidt	93
Giant	88
Yellow Spanish	78
English Morello	76

Average probable error of mean app. 7 per cent.

The cells of the subepidermis are fairly prismatic in outline. The subepidermis is 3 to 7 cells deep.

Table II shows the outstandingly large subepidermal cell size for Yellow Spanish and English Morello which do not crack ordinarily. It appears that firmness and proneness to crack increase with decreasing size and increasing number of subepidermal cells per unit area. Perhaps this finding is in harmony with DORSEY (2) who noted that the tensile strength of the skin of peaches depends upon the subepidermis or deeper layers which show pronounced lateral stretching during growth.

TABLE II

RELATIVE SIZE OF SUBEPIDERMAL CELLS AND DEPTH OF SUBEPIDERMAL LAYER (AVERAGE FROM 5 CHERRIES)

VARIETY	NO. OF CELLS DRAWN FOR MEASUREMENT	AVERAGE RELATIVE SIZE OF SINGLE CELL	AVERAGE RELATIVE DEPTH OF SUBEPIDERMAL LAYER
Lambert	216	100	100
Schmidt	295	128	105
Giant	224	140	135
Napoleon	163	151	129
Bing	239	162	152
Lambert x Schmidt	218	166	141
Yellow Spanish	218	243	152
English Morello	143	350	252

Average probable error of means ± 10 per cent.

The average depth of the subepidermal layer was found to be greatest in English Morello and least in Lambert (table II). The total absolute depth ranges between 0.125 and 0.25 mm. Compared with the total volume of the flesh of the cherry, this magnitude is small. These findings are in harmony with those of KOSMANOFF (6).

Cross sections of the flesh of sour cherries showed an even distribution of large cells measuring from 200 to 350 μ in diameter. The essential point is that most cells of the flesh proper are of the same size. In the firm-fleshed sweet cherries, on the other hand, groups of large cells are regularly interspersed with groups of cells of ½ or ⅓ the diameter of the larger ones, the smaller cells measuring only from 50 μ to 150 μ in diameter. While the largest cells of the flesh of firm-fleshed cherries are fully as large as those of the flesh of sour cherries, the average cell size of the firm-fleshed sweet cherry is only about ½ to ⅔ that of the sour cherry. The vascular bundles are very much more pronounced and cover a much larger area in cross section in the sweet firm-fleshed cherries than in sour cherries.

Physiological considerations

CRACKING OF CHERRIES IN WATER AND IN SUGAR SOLUTIONS

During the season of 1933, ten varieties (Bing, Lambert, Napoleon, Emperor Francis, Giant, Schmidt, Yellow Spanish, Royal Duke, English Morello, and Montmorency) and an F_1 seedling, Lambert × Schmidt, were tested for cracking.

From each of these sorts, 25 selected cherries with the stems attached were submerged for 2, 6, and 18 hours in water and in solutions containing 5, 9.5, 14.1, 18.6, and 23.2 per cent. of sucrose. The cherries were wetted,

TABLE III

SUMMARY OF CRACKING TESTS, 1933*

Variety	Date		Ave. weight	Refractometer reading	Per cent. cracking in water in				Per cent. increase in weight in water in				Remarks
					2 hr.	6 hr.	18 hr.	Index	2 hr.	6 hr.	18 hr.	Index	
			gm.		%	%	%		%	%	%		
Bing	June	22		21.2	0	0	0	0				. .	
		26		22.9	10	20	80	990		.		.	
		28	6.1	20.0	13	19	38	734	2	5	11	178	
		30	6.3	21.0	0	14	57	481	2	7	12	220	Ripe
Napoleon	June	22		16.8	0	0	0	0				..	
		26		18.0	0	0	4	280	.	..		. .	
		28	5.5	19.7	0	0	0	0		...		.. ...	
		30	6.0	17.8	0	24	36	564	2	5	9	172	
	July	3	5.6	21.3	0	32	56	808	1	5	10	155	
		5	5.8	19.7	0	4	52	416	1	5	9	143	
		7	5.8	23.0	0	12	40	436	1	4	8	130	Ripe
		11	5.3	19.3	0	24	76	844	1	4	10	137	
Lambert	June	22		17.8	0	0	0	0	.	.		.. ...	
		26		18.2	0	20	20	400		..	..	......	
		28	4.8	15.4	4	4	4	148	3	4	4	128	
		30	5.5	15.0	0	0	0	0	2	5	8	148	
	July	5	6.5	15.2	0	24	88	928	1	5	7	122	
		11	7.6	21.0	0	52	92	1320	2	4	7	143	Ripe
Emp. Francis	July	7	6.7	21.7	0	12	60	576	2	4	9	141	Ripe
		11	6.0		0	12	8	176	2	5	9	163	
Giant	June	30	4.0	15.1	0	0	0	0	1	8	13	217	
	July	5	4.8	15.2	0	0	0	0	1	5	11	176	
		11	5.0	19.5	0	4	24	224	2	5	9	163	
		14	5.2	20.1	0	8	32	436	3	5	8	160	Ripe
Schmidt	June	22		18.8	0	0	0	0	.	.	.	. .	
		28	4.0	19.8	0	0	0	0	2	4	10	162	
		5	5.1	19.1	0	4	12	136	2	5	9	150	
		7	5.3	20.1	0	0	16	112	2	4	9	144	Ripe
		11	5.3	21.7	0	4	16	164	2	4	8	147	
Yellow Spanish	June	21		17.5	0	0	0	0				. .	
		26	..	22.1	0	10	20	270		.	.	.	Ripe
		28	4.3	22.9	0	0	8	56	2	3	8	124	
Montmorency	July	11	3.6	16.0	0	0	0	0	1	3	4	87	Ripe
		14	3.5	17.0	0	0	0	0	1	2	4	74	

* The results are given in round percentages although decimals were used in calculating the "index" values.

dried, and weighed both before and after submersion. Refractometer readings were made with a Zeiss hand-refractometer on juice expressed from at least 10 cherries. The results of the tests on a few varieties are shown in table III.

Less cracking occurred when the cherries were placed in sugar solutions than in water. No cracking was observed after 18 hours in the 23.2 per cent. sucrose solution. Cracking in most cases increased with progressive ripening. Within any one variety, however, no correlation between the increase in weight of a cherry and cracking could be shown, except in the case of Lambert. No cracking occurred on any cherry lighter than 5 grams in weight. Individual sour cherries weighed approximately 4 grams.

Increase in weight was independent of cracking as expressed by an index of cracking calculated after VERNER and BLODGETT. The index was more than 125 for tests on sweet cherry varieties as compared with an index of less than 88 for samples of Montmorency and 66 for a sample of Royal Duke, both of which showed no cracking. There was a definite water uptake from sugar solutions at all concentrations used.

The refractometer readings on the juice did not show any relation to the cracking of the cherries in different samples as represented by the VERNER and BLODGETT cracking index. Chemical sugar determinations on the juices on which the refractometric readings were taken showed that the readings in the sweet cherries were about 6.5 per cent. higher than the sugar content. HARTMAN and BULLIS state that there is a close relationship between changes in soluble total solids and the changes in total sugars, since the increase in soluble solids is largely an increase in sugars. In the studies of VERNER and BLODGETT the cherries of one variety picked from one tree gave very regular results with regard to increase in total solids, total sugars, and cracking. On the basis of the present results, it is believed that this was a coincidence because both sugar content and cracking commonly increase with advancing maturity.

The increase in weight of the cherries immersed in water showed no correlation to the total solids contained in the juice.

DIFFERENCES IN WATER HOLDING CAPACITY OF THE SWEET AND SOUR CHERRY PULP

Cherries were put through a "Seprosive" which caused thorough crushing of the tissues and also removed the stone and skin by pressing the pulp through a 0.5-mm. sieve. From the resulting mass, which could be well pipetted, 25-cc. samples were placed in wetted folded filter paper inserted into a funnel. The rate with which the liquid passed through the paper was determined by reading at intervals the amount of liquid accumulated

in a graduated cylinder. The results obtained on four varieties of cherries are shown in figure 1.

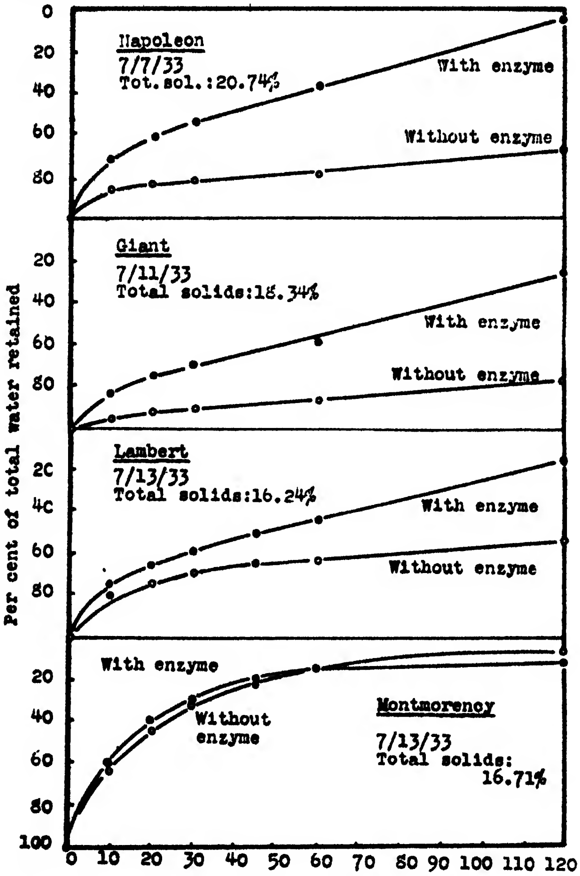

FIG. 1. Water retaining capacity of pulped cherries.

In all of these tests large differences were observed between sweet and sour cherries. As one would expect, the more watery sour cherries after 60 minutes retained only a small fraction of their original water content while the more fleshy sweet cherries yielded barely ⅓ of their original water content.

Further experimental evidence shows that the difference observed was caused by the colloids present. The 25-gram samples of the pulped cherries were treated with 0.5 gram of a mixture of highly active pectic, amylolytic, and proteolytic enzymes for 30 minutes and the mixture poured on to the filter. The water-retaining capacity of the sour cherries, as indicated by the results obtained with Montmorency, showed no significant difference after enzyme treatment. The pulp of the sweet cherries showed a marked

change, however, inasmuch as it filtered much faster than without the enzyme treatment. In a few cases, after longer than 30 minutes of treatment by the enzyme, the water passed through at a rate almost equal to that of the sour cherries.

The importance of these experiments lies in the fact that they show to what a great extent the water-holding capacity is influenced by the colloids present. They prove that the great difference in the water-holding capacity of sweet and sour cherries with only a slightly different content of total solids is caused by the colloids of the sweet cherries.

Some work has been done by the senior author on the differences in the chemical composition between sweet and sour cherries, especially as regards sugar and pectins, which shows that both of these constituents are present in a lower percentage in sour cherries. These results, however, are determinations of a single date only and would mean more if progressive changes in the cherries in several of these constituents were followed throughout the growing season.

Discussion

The morphological studies showed a difference in the subepidermis of cracking and non-cracking cherries. The fact that non-cracking cherries showed relatively large cells in the subepidermis does not explain the phenomenon of non-cracking mechanically. A structure built of smaller units would be expected to have relatively higher tensile strength. The skin (epidermis and subepidermis) of firm-fleshed sweet cherries is probably stronger than that of soft-fleshed sweet and sour cherries, but not sufficiently so to overbalance the relatively higher expansion force of the flesh of the former. In this case also a physical study of this factor should be made upon the isolated tissue but with consideration of the natural forces acting on it *in situ*.

A search of the horticultural literature fails to give any useful information on the permeability of the skin of cherries to water. The morphological studies here reported fail to indicate any difference in the structure which might have a bearing on permeability.

At present it is considered hazardous to correlate cracking in cherries with morphological findings beyond the distinctions which separate firm- and soft-fleshed groups of varieties which, for practical purposes, are obvious without the use of a microtome.

So far as the writers are aware, the swelling of cherries when immersed in water has been attributed solely to osmotic absorption of water through the skin of the fruit. It is stated by Verner and Blodgett, and by Hartman and Bullis, that the highest sugar content of the cherries was not always associated with the most severe cracking. The discrepancy in

this respect is most apparent at full maturity. The changed turgor is said to be responsible for this phenomenon. It is most plausible to presume, however, that absorption by the flesh of cherries and probably also turgor in general is only partly caused by osmosis. The swelling of the colloids present in plant tissues may be responsible for a certain part, if not the major part, of the turgor. The enlargement of certain plant parts, especially of fruits (in a broader sense), is caused mostly by the formation of succulent plant tissues which carry at any time a definite proportion of water. At least a part of the materials present are formed in the fruit itself, causing considerable changes in the water requirements of the tissue at the time of their formation.

It has been suggested that the different behavior of the colloids of sweet and sour cherries might be the result of diversities in their actual acidity. The studies of CALDWELL (1), however, showed that there is not much difference between the hydrion concentration (pH) of sour and sweet cherries. It is more likely that the qualitative and quantitative differences in the colloids are responsible for the differences in cracking.

The water-holding capacity of any plant or plant tissue is, under normal conditions, only partly satisfied. This saturation deficit is indicated by the quick swelling of plant materials not only in water but also in solutions of considerable concentrations (ILJIN 5). This phenomenon can be observed on all varieties of cherries at any stage of maturity because they all take up water when immersed in it.

There are reports in the literature on the high pressures exerted by swelling colloids. Swelling starch, for instance, can produce a pressure of over 2000 atmospheres (KOSTYCHEV 7) and many other colloids show a similar behavior.

It is considered likely that attraction for water by the cherry fruit is caused by the constituents of the cell walls. As the morphological studies have shown, these materials are present in larger quantity in the firm-fleshed sweet cherries. Since the sugar content of firm sweet, soft sweet, and sour cherries is not appreciably different, and since the attraction of the pulp for water does vary, this variation is held to be due to differences in the colloid content of the fruit and is considered responsible for the cracking of cherries.

Summary

1. The size of the subepidermal cells in cherries which are prone to crack is appreciably smaller than in those which do not crack, the number of subepidermal cells per area being correspondingly larger.

2. In cherries which tend to crack, the large parenchymatous cells of the flesh are more frequently interspersed with smaller cells, thus decreas-

ing the average cell size considerably. In the non-cracking types examined, the average cell size of the flesh is relatively larger and more uniform.

3. The size of cells of the epidermis is not correlated with proneness to crack. Thickness of the inner wall of the epidermis is positively correlated with cracking.

4. Both the rate and extent of cracking of cherries are lessened when the fruits are immersed in sugar solutions instead of in water. No cracking could be observed on cherries immersed in 23.2 per cent. sucrose solution, and only 19 per cent. of the samples which cracked in water cracked when immersed in 18.6 per cent. sucrose solution for 18 hours.

5. All cherries used in this study took up water when immersed in water or in sucrose solutions. Although no correlation between water uptake and cracking could be shown within one variety, the cracking and non-cracking cherries formed two distinctly separate groups, the water uptake in the latter group being much lower. This fact indicates a difference in saturation deficiency of the cherries of the two groups.

6. It is suggested that the cracking of cherries is caused by the forces of the swelling colloids of the flesh rather than by osmotic forces.

7. In the foregoing statements, "cherries prone to crack" is roughly equivalent to hard-fleshed varieties, "cherries not prone to crack" grossly corresponds to soft-fleshed varieties.

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STREAMLINE FLOW AND THE MOVEMENT OF SOLUTES IN THE TRANSPIRATION STREAM

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Introduction

The classic experiment of Askenasy (**1, 2**), illustrating the pull exercised by an evaporating water surface, consists, as is well known, of a thistle funnel closed at the wide end by a plug of plaster of paris, made air-tight, if necessary, by ringing with gold-size. The funnel is filled with well boiled distilled water, and inverted into a vessel of mercury. As the water evaporates from the upper surface of the plaster plug the mercury rises *pari passu* at the lower end of the tube, and might be expected to reach eventually barometric height, less the vapor pressure at the prevailing temperature. Actually the surface tension and the molecular cohesion of the water draw the mercury to much greater heights. Askenasy observed a rise of 89 cm. at a barometric pressure of 73.5 cm. Ursprung, in a series of papers (**7, 8, 9, 10, 11**), made more extensive observations and obtained a rise of 135 cm. above barometric height, in glass tubes, and of double barometric height in liane stems used as tubes. In all cases, bubbles are formed eventually and the cohesion breaks down.

The rate at which the mercury rises depends, of course, primarily on the rate of evaporation and secondarily on the ratio between the area of the evaporating disc and the cross section of the tube. The rate, however, is not exactly equivalent to the rate of removal of the water. Nordhausen (**3**) has shown that an evaporating surface can raise water from a vacuum, *i.e.*, develops beneath it a negative pressure of at least one atmosphere. Our own experimental comparisons of the weight/volume ratio during the rise of the mercury column showed a diminution in the density of the water of as much as 10 per cent., so that as the experiment proceeds an increasing negative pressure develops in the water, long before barometric height is reached, which tends to withdraw the water menisci from the pores of the evaporating surface and thus check the rate of flow.

The existence of this negative pressure can readily be shown by using a thistle funnel with plaster plug, the stem of which dips into mercury in a closed filter flask. The flask is partly evacuated and time is then allowed for a negative pressure to establish itself and for the mercury to rise a short distance in the stem of the funnel. On admitting air to the flask the mercury rises with a sudden bound upward, often to twice its previous height, in replacement of the contracted water column.

Procedure

If, during the course of the ASKENASY experiment, a small amount of a strongly colored solution is introduced at the bottom of the tube by means of a curved pipette, so that the solution floats above the mercury, it will be observed that the upper surface of the colored layer does not rise as a level surface, but is drawn up in a delicate axial thread of color which advances at a surprising speed and reaches the top of the tube in a few minutes, even at low rates of evaporation. An array of dyes was tested in this way and it was found that the rate of rise was independent of the molecular weight of the dye used, and is therefore not a simple diffusion phenomenon. Moreover, the characteristic movement is absent in tubes sealed at the top.

For our experiments the original arrangement of the apparatus was modified only by the addition of an adiathermic shield, formed of a wide glass sleeve filled with a saturated solution of nickel sulphate. As each funnel has its own characteristics, compared tests were always made with the same funnel.

Observations

The apex of the column is sharply defined and usually obtuse. The latter point is striking. The appearance is different from that which one sees on applying a hydrostatic suction to the upper end of the water column when the color is drawn up axially in fine vanishing point. In this latter case, neutral red introduced over a 2-inch column of mercury forms a dense layer, with no streaming, until gentle suction is applied by opening a capillary siphon attached to the top of the tube. As soon as flow starts the color is immediately drawn out along the median line, forming a narrow cone with straight sides, which lengthens out so long as flow continues. When the siphon is closed the flow stops and the color sinks slowly back by gravitation into a uniform diffusion gradient. Left thus undisturbed the color took two weeks to reach the top of a 2-foot tube.

The rates of rise observed for the axial column of color are given in table I.

The rates of flow are linear until the top of the tube is reached, irrespective of the dye used.

The air temperature of the laboratory varied throughout the experiments, but the mean deviation of the averages for each group of tests from the average for the whole series was only 1.0° C., so that temperature changes cannot account for the observed differences.

Although experiments with sealed tubes and siphons, referred to above, show that the phenomenon is not one of diffusion pure and simple, there is a general relation between the diffusibility of the dyes and the rate of

TABLE I

Rise of dyes in Askenasy tubes*

(Averages of three observations in each case)

Dye used	Molecular weight	Average time to rise 10 inches (half length of tube)
Thionin	263	24 minutes 31 seconds
Neutral red chloride	288	12 minutes 53 seconds
Methylene blue	319	10 minutes 47 seconds
Safranin	350	6 minutes 25 seconds
Bismarck brown	418	3 hours to 6 inches only
Janus green B	422	20 minutes 39 seconds
Methyl violet 6B	482	14 minutes 45 seconds
Benzopurpurin	628	No rise
Congo red	696	42 minutes to 4 inches only
Trypan red	766	60 minutes 0 seconds
Trypan blue	966	No rise

* Height of mercury in each case before introduction of dye, 1.4 inches.
Height of mercury after introduction of dye, 1 inch.
Quantity of dye (0.5 per cent. solution) introduced, 0.3 cc.
Mean air temperature, 18.4° C.

rise. Some of the dyes used are colloidal or semi-colloidal, and their solution aggregates are apparently too heavy to be raised in the flow lines of these tubes. Such are benzopurpurin, congo red, and trypan blue, as table II shows.

Theoretical considerations

The comparative rate of rise, however, is only of secondary importance in consideration of the remarkably rapid movement of practically all the

TABLE II

Comparison of rate of rise and diffusibility

Dyes in order of rate of rise	Order of diffusibility into 10 per cent. gelatin
1. Safranin	1
2. Methylene blue	2
3. Neutral red chloride	4
4. Methyl violet	6
5. Janus green	5
6. Thionin	3
7. Trypan red	8
8. Bismarck brown	7
9. Congo red	10
10. Trypan blue	9
11. Benzopurpurin	11

dyes, in the axial line. This appears to be due to the actual vertical flow of the water column and finds its explanation in the classic researches of REYNOLDS (5) on water flow in parallel-walled channels.

In REYNOLDS' experiments the colored fluid was introduced into the water current from a fine jet near the inlet end of the flow tube. At low speeds the color was drawn out into a fine axial thread, but as the rate of water flow increased the thread showed disturbance, due to eddies, near the outlet. This turbulent flow gradually approached nearer to the inlet end, until at a given speed the whole flow became turbulent. If the rate of flow was again reduced, it was found that the turbulent flow again gave place to linear flow at a fairly definite velocity, called the "critical velocity."

No sign of turbulent flow was observed in our experiments. This was according to expectation, for the flow due to evaporation is extremely slow and well below the critical velocity.

We may conclude that the flow of water in xylem vessels, in common with that in the experimental tubes, is purely of streamline type. The following considerations will make this clear.

In streamline flow, the inertia forces are small compared with the tangential frictional forces between neighboring layers of fluid. Calculations of the rate of flow, based purely on viscosity, agree so well with experimental observation that the reverse calculation is used as a standard method for measuring viscosity. When a fluid is moving over a smooth plane surface, the layer actually in contact with the surface is at rest, and each successive layer outward moves with greater velocity, *i.e.*, there is a velocity gradient normal to the surface. If v is the velocity parallel to the surface at any distance, y, measured perpendicular to the surface, then the velocity gradient at the surface is given by $\frac{dv}{dy}$.

Applying this to flow in pipes, REYNOLDS found that the critical velocity, v_c, occurs under all conditions at a constant value of the ratio $\frac{v_c \delta \rho}{\mu}$, where δ = the diameter of the pipe, ρ = the density of the fluid, and μ = the viscosity coefficient. This ratio has come to be known as the REYNOLDS number. It is valid for all conditions of flow and with all fluids, and its average value is 2000. As a ratio it is non-dimensional.

The mean critical velocity for water is therefore $2000 \times \frac{\mu}{\delta\rho} = \frac{26}{\delta}$ at 15° C., where δ is the diameter of the pipe in centimeters. The critical velocity is therefore inversely related to the diameter of the tube. Assuming a diameter of 1.0 mm. for a xylem vessel, as an extreme case, the average critical velocity for water at 15° C. in such a tube would be 2.6 meters per second, which is well above physiological limits.

If, therefore, we are entitled to assume a condition of streamline flow as existing normally in xylem channels, then the difference between the axial and the average velocities in the moving columns of water in the vessels becomes a matter of considerable physiological importance.

OWER (4), discussing REYNOLDS' work, points out the conditions for the determination of this difference. Consider any cylindrical element of radius r, assumed to lie in the axis of the pipe, the upstream end being denoted by A and the downstream end by B, the distance between them being dl. The pressure on $A = p$ and on $B = p - dp$. The case is the same whether a positive pressure be applied to A or a negative pressure to B. The pressure difference maintaining the motion of AB is then $\pi r^2 dp$. This quantity must equal the retarding force of fluid viscosity acting on the exterior of the cylinder, that is to say $2\pi r dl \mu \frac{dv}{dr}$, when $v =$ the velocity of flow at radius r.

Therefore

$$\pi r^2 dp = -2\pi r dl \mu \frac{dv}{dr} \quad \ldots\ldots\ldots\ldots (1)$$

and

$$\frac{dp}{dl} = -\frac{2\mu}{r}\frac{dv}{dr} \quad \ldots\ldots\ldots\ldots (2)$$

As the pressure does not vary across the section of the cylinder, $\frac{dp}{dl}$ is constant for given conditions, and as velocity in streamline flow varies only with r, then $\frac{2\mu}{r}\frac{dv}{dr}$ is a function of r only.

Now $\frac{dp}{dl}$ may be written as equal to $\frac{p_1 - p_2}{l}$ where p_1 and p_2 are the respective pressures at each end of l.

Therefore $\frac{p_1 - p_2}{l} \cdot r dr = -2\mu dv \ldots\ldots\ldots\ldots (3)$

Integrated from $r = 0$ to $r = R$ the full radius of the tube, $\frac{p_1 - p_2}{l}\frac{2}{R^2} = -2\mu(V_R - V_a)$, when $V_a =$ the velocity at the axis, and, as the velocity at the wall of the tube is zero, $V_R = 0$ and

$$\frac{p_1 - p_2}{l} = \frac{4\mu V_a}{R^2} \ldots\ldots\ldots\ldots (4)$$

Thus

$$V_r = \frac{p_1 - p_2}{4\mu l}(R^2 - r^2) \ldots\ldots\ldots\ldots (5)$$

which shows that the distribution of velocity according to radius is parabolic across the tube section.

Also, from equation (4) we have

$$V_a = \frac{p_1 - p_2}{4\mu l} \cdot R^2 \qquad (6)$$

From the classic formula for absolute viscosity: Volume flowing in unit time $= \frac{\pi R^4 (p_1 - p_2)}{8 \mu l}$ and volume flowing in unit time also $= \pi R^2 V_m$ where V_m is the mean velocity of flow.

Therefore the mean velocity, $V_m = \frac{p_1 - p_2}{8 \mu l} R^2 \qquad (7)$

so that the axial velocity (equation 6) is twice the mean velocity.

Even where the absolute amount of flow is slight, there will be a relatively rapid movement of the axial column of liquid, as is evidenced by the rapid ascent of the dye solution in our experiments. Comparison of observations, made with the same apparatus, of the halfway rise of neutral red chloride at varying rates of evaporation, as measured by the number of minutes taken by the mercury to rise from zero to 1 inch, show, however, that the relationship is not so simple as the preceding reasoning suggests. According to equation (6) the relationship of axial velocity to pressure in the water column is directly linear. When the times of rise are plotted against rates of evaporation, however, the points show, although with considerable scatter, a convex distribution with a mode near the 1000-minute line, under the conditions of the experiment. On either side of this line the rate of dye ascent increases, with the paradoxical result that increasing the rate of evaporation from say 2000 to 1000 minutes per inch of mercury may have the effect of decreasing the rate of dye ascent, and vice versa. Any movement toward the mode, from either direction, is a move toward slower rates of ascent.

It is also found that extreme reduction in the rate of evaporation, short of absolute stoppage, has comparatively little effect on the rate of dye ascent, although it reduces the amount of dye raised. Thus, in one case where the mercury took 8 days (11,150 minutes) to rise from 0 to 1 inch, neutral red, when introduced at the end of this period, rose 10 inches in 10 minutes and 30 seconds.

In the other direction, the rate of ascent increases rapidly as the evaporation increases, until a point is reached, not very exactly defined but in the region of the 200-minute rate, where the phenomenon appears to cease altogether, although the rate of water movement is still considerably below the critical velocity. This result, at first sight surprising, may be deduced from the consideration of equations (5) and (6).

From equation (5) it will be seen that as $p_1 - p_2$ increases V_r will increase toward the limiting case, determined by the REYNOLDS number, where streamline flow ceases. At this point $v_r = v_a$ and the radial gradient of velocity disappears. As this condition is approached r^2 diminishes and r

therefore becomes vanishingly small some time before the limit is reached; that is to say, the axial movement is restricted to a line of water too tenuous to draw up any observable amount of dye. The surface of the dye layer will therefore appear to remain undisturbed until true turbulent flow sets in. This corresponds presumably to the region of instability found by STANTON and PANNELL (6).

Conversely it may be seen that as $p_1 - p_2$ diminishes, with lower rates of evaporation, the consequent diminution of v_r may be compensated by the diminution of r^2. Reference to equation (1) shows that r is a more important factor in the equilibrium of force against viscosity than v, the velocity, and that equilibrium will be more economically maintained by changes of r than of v. With diminishing r one might therefore expect v to be maintained down to low values of $p_1 - p_2$.

A point of particular interest is that, in the evaporating system of the ASKENASY tube, there may be a movement of material against a concentration gradient. If a small quantity of dye be introduced at the base of the tube the axial current will shortly draw it all to the top of the tube, where it accumulates, so that the latter fractions of the dye material are moving toward a region of concentration.

Discussion

The application of these ideas to the conditions of water flow in the xylem looks, in the first place, to the cases where, under conditions of very low transpiration, such as obtain in very humid and sometimes also in very dry situations, there is little or no negative pressure and the cell elements are full of water. In any such case, short of complete hydrostasis, solutes will travel up the axes of the water channels at speeds considerably greater than the total measured water flow would suggest.

Even where air bubbles are present in the vessels, the free water-air surface will still travel at a rate at least twice the mean rate of flow, which will itself be locally accelerated in such conditions by the restriction of the available channel.

Observations at reduced rates of evaporation suggest that under such circumstances a tension may develop which leads to axial rates in excess of twice the mean velocity. In the case just mentioned, for example, where mercury took 8 days to rise 1 inch, the dye rose 10 inches in 10½ minutes. Observations made under constant conditions of evaporation, but starting with the mercury at varying heights in the tube, also show a consistent increase of velocity of axial flow as the mercury level rises.

The rate of flow is also slightly greater, under all conditions, in the upper part of the tube, but most markedly so when evaporation is reduced, that is to say under those circumstances where local tension is most likely to develop. The state of tension, most accentuated at the axis, reduces the

density and hence the viscosity, to which the velocity of flow is inversely related.

Summary

The problem of the diffusion of solutes in plants with very low rates of transpiration may be explained by consideration of the radial velocity gradient due to streamline flow in tubes.

With low rates of flow, there is a parabolic velocity gradient normal to the walls of the tube, and an axial current of at least twice the mean velocity of flow. Experiment shows that these conditions are maintained, and even exaggerated at very low rates of water movement, and only disappear, in the one direction at complete hydrostasis and in the other direction at a velocity some way short of the beginning of true turbulent flow, which latter cannot, however, normally occur in the transpiration channels. Movement of the solute under these conditions may take place toward a region of concentration.

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UNFREEZABLE AND FREEZABLE WATER EQUILIBRIUM IN PLANT TISSUES AS INFLUENCED BY SUB-ZERO TEMPERATURES[1]

GLENN A. GREATHOUSE

Introduction

It is commonly accepted that with biocolloidal systems there is a definite temperature at which all of the freezable water will be frozen (**7**, **11**, **6**, and others). This temperature has been arbitrarily selected as about $-18°$ to $-20°$ C. In studying one phase of a problem on winter hardiness in red clover roots, it was desirable to determine the amount of unfreezable water in these tissues. Thus an opportunity was afforded to test the preceding hypothesis with regard to this tissue by determining the amount of water which does not freeze at temperatures of $-10°$ to $-50°$ C. Potato tubers were also included in the study.

Review of literature

ST.JOHN (**9**) made a study of the unfreezable and freezable water equilibrium of the thick portion of egg white, employing the method of RUBNER (**7**) as modified by THOENES (**11**) and ROBINSON (**6**). He found that at $-5°$ C., 80 per cent. of the water present was unfreezable, while at $-10°$ C. only 35 per cent. remained unfrozen. Determinations at $-12.5°$ C. showed 25.8 per cent. of the water unfreezable. From this temperature on through $-35°$ C. he found a flat straight-line curve indicating that no more of the water was frozen at a temperature of $-35°$ C. than at $-12.5°$ C.

JONES and GORTNER (**3**), using the dilatometer method on gelatin solutions, found that all of the freezable water was crystallized out at $-6°$ and no additional amount was removed at a temperature of $-50°$ C. With a 2 per cent. solution the percentage of total water unfreezable was 9.35. At a temperature of $-44.4°$ C. the unfreezable water percentage was exactly the same as at $-6°$ C. They state that animal and plant tissues and related systems would probably behave like the gelatin system. It does not necessarily follow, however, that such would be the case for plant tissues.

JONES and GORTNER'S (**3**) data on inorganic hydrogels indicate that there was no temperature within the range of 0.0° to $-50°$ C. at which a lower temperature did not cause the crystallization of additional quantities of ice.

[1] The writer expresses his appreciation to Dr. E. A. HOLLOWELL, of the Forage Crop Division, U. S. D. A., for furnishing the clover seed used in this investigation and for his valuable suggestions in culturing red clover plants.

Methods

A variety of methods have been used by investigators for determining the ratio of unfreezable to freezable water in organic materials. Of these the early method of MÜLLER-THURGAU (5), later modified by RUBNER (7), THOENES (11), ROBINSON (6), ST.JOHN (9), SAYRE (8), and MEYER (4), was chosen. This is the calorimetric or heat of fusion method. It has been modified slightly to meet the requirements of this particular problem. The symbols and equation used follow in general those developed by previous investigations.

The tissues used in the unfreezable water determinations were put through a Nixtamal mill. Portions were then taken for total and freezable water determinations, the total moisture being determined in a vacuum oven at 80° C. and 3 to 4 cm. pressure. The total moisture value was used in the unfreezable water calculations instead of the moisture determinations on the same following the calorimetric determinations, as has been done by a number of previous investigators.

For the freezable water determination, 8 to 10 gm. of tissue were placed in a tared tinfoil cup, similar in construction to that used by ROBINSON (6), which was then placed in a tared weighing bottle. The whole was weighed accurately and placed in the low temperature cabinet at the desired subzero temperature. For the temperatures of $-10°$ to $-23°$ C. a specially constructed cabinet was used. For temperatures of $-25°$ to $-50°$ C. an alcohol bath, to which dry-ice was added to give the desired low temperature, was employed. The inner container of tinfoil is essential in weighing the tissue and in making a quick transfer of the frozen tissue to the calorimeter. It likewise aids in preventing (1) floating of tissue on surface of water in calorimeter, (2) evaporation of water from tissue during weighing, etc., and (3) heat of solution of any soluble substances while in the calorimeter. The outer container aids in the identification and transfer of the tissue from the sub-zero temperature to the calorimeter with the minimum of time and exposure to room temperature.

The determination of unfreezable water by the calorimetric method requires the establishment of a stable phase equilibrium at the desired sub-zero temperature. The value for unfreezable water varies with temperature, within limits, and it is important to maintain the desired freezing temperature constant, preferably with $\pm 0.25°$ C., for a period of hours. The requirement of $-10°$ to $-23° \pm 0.25°$ C. was fulfilled in a special low temperature unit constructed by the Freas Electric Company.

The freezable water samples were allowed to remain in the cabinet for 10 to 12 hours. Triplicate samples with different time intervals indicated that the different phases had attained an equilibrium in this period.

The calorimeter consisted of a highly evacuated silvered Dewar flask, closed with a cork 3 cm. thick. Two holes were placed through the cork, one for the thermopile or Beckmann thermometer and the other for a spiral glass stirrer. Proper stirring of the liquid is essential to establish an equilibrium between the material and the liquid of the calorimeter.

Exactly 300 gm. of distilled water were placed in the calorimeter. As soon as the water had come to a temperature equilibrium with the calorimeter walls, glass stirrer, and thermo-elements, the frozen sample was introduced and the new equilibrium determined. The time required to reach equilibrium for the 8 to 10-gm. sample was 8 to 10 minutes, with a constant rate of stirring. The temperature was measured by a Beckmann thermometer or by the use of a 15-junction thermopile attached to a Leeds and Northrup type K potentiometer. The thermopile was constructed of 28 gauge copper and constantan wire. It was possible with this thermopile and accessory equipment to measure 0.001° C.

With the addition of the frozen sample to the water of the calorimeter there is a fall in temperature. In this investigation the heat lost by the water in the calorimeter is used in warming six different component parts of the system. Each of these quantities may be expressed as follows:

$$
\begin{array}{ll}
(1) & W_t \; S_t \; (T_e - T_s) \\
(2) & W_d \; S_d \; (T_e - T_s) \\
(3) & W_b \; S_b \; (T_\triangle - T_s) \\
(4) & W_i \; S_i \; (T_\triangle - T_s) \\
(5) & W_w \; S_w \; (T_e - T_\triangle) \\
(6) & H_f \; W_i
\end{array}
$$

W_t = weight of tinfoil cup;
W_d = weight of dry matter in sample;
W_b = weight of unfreezable water in sample;
W_i = weight of ice in sample;
W_w = total water in sample;
S_t = mean specific heat of tinfoil for temperature range used;
S_d = mean specific heat of dry matter for temperature range used;
S_b = mean specific heat of unfreezable water for temperature range used;
S_i = mean specific heat of ice for temperature range used;
S_w = mean specific heat of water for temperature range used;
H_f = heat of fusion of ice (79.75 calories) at $T_\triangle$;
T_e = equilibrium temperature;
T_s = sub-zero temperature of sample;
$T_\triangle$ = freezing-point of water in plant sap.

It is necessary to determine a correction factor for the calorimeter, since the walls of the calorimeter, stirrer, etc., absorb heat from the water the same as the components in the system. This correction factor was obtained by placing 8–10 gm. of water in the tinfoil cups, freezing at the desired temperature, and then transferring to the water of the calorimeter and determining how much heat was necessary to melt the ice and raise the temperature of the resulting water to the equilibrium temperature of the system. The values for the correction factor were plotted for the different sized samples. Thus the factor could easily be obtained for the sample that varied from 8 to 10 gm. For an 8-gm. sample the correction factor was found to be 1.085.

It is evident that the loss of heat energy in terms of calories by the water in the calorimeter may be expressed mathematically as follows: (7) $FNS_w (T_o - T_e)$

where F = calorimetric correction factor;

N = number of grams of water in calorimeter (300 gm.);

S_w = specific heat of water for temperature range used $(T_o - T_e)$;

T_o and T_e = original temperature of water and equilibrium temperature respectively.

Specific heat determinations were made on the dried tissue, following the procedure previously outlined, except that benzene was substituted for the water in the calorimeter. It was found that the use of a liquid of lower specific heat increased the accuracy of the determination. The values used for the specific heat of pure benzene were secured by plotting a curve for the values as given in the International Critical Tables (**12**).

The specific heat values for ice were taken from DICKINSON and OSBORNE (**1**). The values for water above zero were taken from the Handbook of Chemistry and Physics (**2**). The specific heat values for water below zero were obtained by extending the curve of values from 0° to 30° C. as a straight line, assuming that the specific heat of water continues as a linear function, as has previously been done by SAYRE (**8**). It is assumed that the specific heat of unfreezable water is the same as that of freezable water at the same temperature.

Combining quantities 1, 2, 3, 4, 5, 6, and 7, equating and solving for W_1, the final complete formula becomes:

$$W_1 = \frac{FNS_w (T_o - T_e) - \quad W_t S_t (T_e - T_s) + W_d S_d (T_e - T_s) + W_w S_w (T_e - T_s)}{H_f - \quad (S_b - S_1)\ (T_\triangle - T_s)}$$

The values for T_s and $T_\triangle$, being below zero, are indicated in the formula by a negative sign. The unfreezable water is determined by subtracting the freezable water from the total water, thus $W_b = W_w - W_1$.

Results

The temperatures used were from – 10° to – 50° C. by five degree steps. In addition measurements were made at – 22° C. The physical chemist (**10**) considers – 22° C. as the minimum freezing-point of pure water under any set of equilibrium conditions. The data presented in table I show the variation in results for typical triplicate samples and indicate the accuracy of the method.

TABLE I

PERCENTAGES OF UNFREEZABLE WATER IN UNHARDENED CLOVERS AT DIFFERENT SUB-ZERO TEMPERATURES (OHIO VARIETY)

SUB-ZERO TEMPERATURE °C. ± 0.25	UNFREEZABLE WATER	
	TOTAL WATER	TOTAL SOLIDS
	%	%
– 10	20.502	103.936
	20.548	103.988
	20.536	103.926
– 15	12.011	60.786
	13.940	70.543
	14.501	73.387
– 20	8.672	43.883
	9.555	48.353
	9.307	47.101
– 22	7.057	35.716
	7.925	40.106
	7.279	36.853

Table II presents representative data that have been calculated from measurements made upon clover and potato tissue when exposed to temperatures from – 10° to – 50°. It will be noted that as the temperature decreases from – 10° to – 50° C. (table II, A and C) in the case of the unhardened red clover root tissue, the unfreezable water value also decreases. A similar condition can be observed on the hardened-off clover root tissue between – 15° and – 20° C.; however, from – 20° to – 25° C. the unfreezable water value remains nearly constant. Part C of table II presents data to show the influence of very low temperatures upon the unfreezable water values. It will be noted that there is a lowering of the unfreezable water fraction with each five degree fall in temperature. This is especially marked between the temperatures of – 10° to – 30° C. The rate of decrease becomes slower with the lower sub-zero temperatures. Potato tissue (table II) behaves similarly to clover root tissue, with the exception that the unfreezable water ratio is shifted slightly farther to the right.

TABLE II

INFLUENCE OF SUB-ZERO TEMPERATURES ON PERCENTAGE OF UNFREEZABLE WATER IN CLOVER AND POTATO TISSUE

Kind of tissue	Sub-zero temperature °C. ± 0.25	Unfreezable water		Total moisture
		Total	Total solids	
		%	%	%
Red clover roots— A: Ohio, unhardened, same age as B	– 10	20.53	103.95	83.50
	– 15	13.48	68.24	
	– 20	9.18	46.45	
	– 22	7.42	37.56	
B: Ohio, hardened, same age as A	– 15	15.43	78.06	82.30
	– 20	13.16	68.17	
	– 22	14.03	71.04	
	– 25	13.65	69.09	
C: Unknown variety grown in field, unhardened	– 10	14.47	72.16	81.81
	– 15	9.43	52.04	
	– 20	7.36	38.66	
	– 22	5.28	24.73	
	– 25	2.66	14.23	
	– 30	1.99	11.76	
	– 35	2.11	12.84	
	– 40	1.95	11.54	
	– 45	1.50	10.63	
	– 50	1.43	10.59	
D: Potato (Irish Cobbler) tubers	– 10	11.05	47.31	81.07
	– 15	9.16	38.18	
	– 20	7.75	33.18	
	– 23	5.59	23.96	

Discussion

A number of investigators have suggested that water binding in biological materials follows an adsorption reaction. The results of this investigation indicate more nearly an equilibrium between the external force (low temperature) and internal forces of the tissue.

The writer's experimental data with plant tissues exposed to sub-zero temperatures do not follow exactly the generalizations of JONES and GORTNER (3) on gelatin, or that of ST.JOHN (9) on egg white. The results of table II, parts A, C, and D, follow more closely the data that have been secured by different investigators on inorganic hydrogels; whereas the data in table II, B, more nearly coincide with the findings of JONES and GORTNER on gelatin and ST.JOHN on egg white. However, the constancy of unfreezable water values occurs at lower sub-zero temperature.

There are indications that the same tissue (table II, A and B), when grown under different conditions, as temperature, will produce entirely different values for the unfreezable water value when the determinations are made at – 22° or – 25° C.

Summary and conclusions

1. The hypothesis of RUBNER (7), THOENES (11), ROBINSON (6), and others that biocolloidal systems have a definite temperature (– 18° to – 20° C.) at which all of the unfreezable water will be frozen has been tested for plant tissues. This hypothesis is not supported by unhardened clover roots, but seemed to be by the hardened clover root tissue. The unfreezable water values decreased 1.40 per cent. for the cold hardened red clover roots with the lowering of the temperature from – 15° to – 22° C., whereas the unfreezable water values of the unhardened root tissue decreased 6.02 per cent. over the same temperature range.

2. With another lot of unhardened red clover root tissue there was a decrease in the amount of the unfreezable water expressed as the percentage of the total water, from 14.47 to 1.43 per cent. with the decrease of the temperature of – 10° to – 50° C.

3. Potato tissue gave similar results to those of unhardened clover root tissue, with the exception that the unfreezable water values were lower for similar sub-zero temperatures.

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HORMONES IN RELATION TO ROOT FORMATION ON STEM CUTTINGS[1]

WILLIAM C. COOPER

(WITH FOUR FIGURES)

Experimental work with one of the root-forming hormones, β-indolyl-acetic acid, has for the most part been limited to root formation on pea cuttings. This paper presents results obtained from applying the hormone to stem cuttings of other plants with the purpose of determining its practical value in propagation by cuttings.

Pure synthetic β-indolyl-acetic acid was used in all of the experiments and was applied to the cuttings by the lanolin method of LAIBACH (2). One part of the hormone was mixed with 2000 parts of pure lanolin, in which it is soluble. A small portion of this paste—roughly about 10 mg.—then was smeared on a small area, about 0.5 × 0.125 inch, on one side of the cutting near the top which had previously been scraped to remove the epidermis and outer cortical layers.

Lemon experiments

Twigs of mature wood of the current and of the previous year's growth were taken from a Eureka lemon tree on April 30, 1935, and were made into 80 cuttings about 5 inches long and with 4 or 5 leaves. The cuttings were divided into 10 lots and treated as shown in table I. Girdling, when used, consisted of removing a complete ring of bark about 1 cm. in width at about 1.5 in. from the base of the cutting. All leaves below the girdle were removed and, in the case of the treated cuttings, the hormone was applied above the girdle. Only the portion of the cutting below the girdle was placed in the sand.

The results presented in table I and figures 1, 2, and 3 show that, in the case of ungirdled cuttings with leaves, the hormone not only caused greatly increased rooting as compared with controls, but accelerated rooting considerably. There was no very significant difference in the total number of roots formed by apical and basal cuttings of terminal growth and by cuttings from the previous season's growth; apical cuttings of terminal growth, however, did form roots sooner than the other types of cuttings.

As shown in table I, the hormone not only caused increased production of roots on cuttings with leaves, but also caused root formation on cuttings without leaves, while on controls without leaves, no roots were formed.

[1] These investigations were conducted at the William G. Kerchoff Laboratories of Biology, California Institute of Technology, and the writer is indebted to the workers in these laboratories for supplying the hormone and other facilities.

TABLE I

ROOTING EXPERIMENTS WITH EUREKA LEMON CUTTINGS* WITH AND WITHOUT THE APPLICATION OF ROOT-FORMING HORMONES

LOT	TYPE OF CUTTING	LEAVES	HORMONE	GIRDLED	NO. OF CUTTINGS	AVERAGE NO. OF ROOTS PER CUTTING: AFTER 2 WEEKS	AFTER 3 WEEKS	AFTER 4 WEEKS
1	Tip of terminal growth	+	+	−	10	0.1	2.6	5.0
2	" " "	+	−	−	5	0.0	0.6	1.6
3	Base of terminal growth	+	+	−	10	0.1	1.4	6.0
4	" " "	+	−	−	5	0.0	0.0	1.1
5	Previous season's growth	+	+	−	5	0.0	2.0	4.2
6	" "	+	−	−	5	0.0	0.0	0.8
7	Base of terminal growth	−†	+	−	10	0.0	1.1	1.6
8	" " "	−	−	−	10	0.0	0.0	0.0
9	" " "	[illegible]	+	+	10	0.0	0.0	0.4‡
10	" " "	+	−	−	10	0.0	0.0	0.2§

* Set in sand in sash-covered propagating frame in greenhouse and shaded with burlap.
† Leaves removed at time of placing in sand.
‡ All roots occurred on two cuttings which had formed callus across girdle.
§ All roots occurred on one cutting which had formed callus across girdle.

FIG. 1. Rooting of hormone-treated apical cuttings from terminal growth of the lemon.

HALMA (1) reported the rooting of 4 lemon cuttings without leaves out of 27 tested, but stated that the amount of roots produced was insignificant. This, however, was not the case with hormone-treated leafless cuttings, because, as shown in figure 4, a really significant number of roots was obtained. This is considered as good evidence that the formation of roots is influenced by a supply of root-forming substance as well as by a supply of food material. Leafy control cuttings which rooted probably obtained their supply

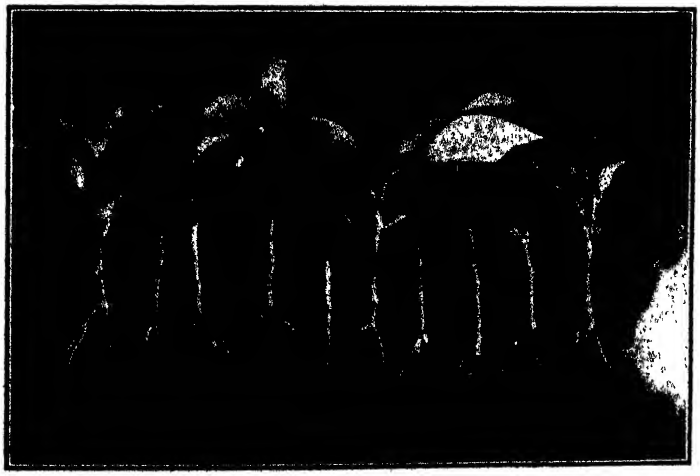

FIG. 2. Rooting of hormone-treated basal cuttings from terminal growth of the lemon.

FIG. 3. Rooting of control cuttings from apical and basal portions of terminal growth of the lemon: apical on right, basal on left.

of root-forming substance from the leaves where presumably it is manufactured.

It may also be noted from table I that girdled cuttings with hormone applied above the girdle did not form roots except in two instances where a callus had grown over the girdle and laid down new phloem tissue across the girdle. This evidence indicates that the hormone is transported in the phloem. Had the stem below the girdle been supplied with hormone, it

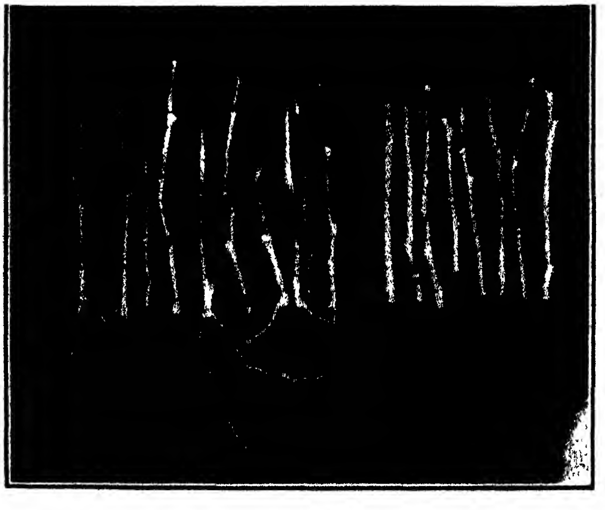

FIG. 4. Rooting of leafless lemon cuttings: right side, untreated; others, hormone treated.

would have rooted, as the results with leafless cuttings showed that stems without leaves will root when a supply of hormone is available.

These results with the rooting of lemon cuttings by hormone were so encouraging that experiments are now in progress with oranges and grapefruit, which are known to be more difficult to root than the lemon.

Experiments with cuttings of other plants

Results of experiments with cuttings of *Lantana*, fig, *Acalypha*, and *Tradescantia* are shown in table II. The *Lantana* cuttings were about 2 inches long and consisted of one internode and one node (at top) with two subtending leaves. They were placed in sand in an outdoor sash-covered frame on March 10, 1935. The hormone-treated cuttings produced a much larger number of roots than the untreated cuttings. Most of the roots on the untreated cuttings appeared at the base of the cutting, while on the treated cuttings roots were scattered all over the internode, appearing mostly at the point of application of the hormone, even when the hormone was applied to a portion of the cutting above ground.

Acalypha cuttings without leaves, planted in sand with bottom heat on March 19, 1935, had over six times as many roots on treated cuttings as on controls. As with *Lantana*, a large number of roots on the treated cuttings came out near or at the point of application of the hormone.

The results with the fig presented in table II were obtained from dormant cuttings placed in sand with bottom heat on February 19, 1935.

TABLE II

ROOTING EXPERIMENTS WITH CUTTINGS OF OTHER PLANTS WITH AND WITHOUT THE APPLICATION OF ROOT-FORMING HORMONES

PLANT	WEEKS AFTER PLACING IN SAND*	AVERAGE NUMBER OF ROOTS PER CUTTING	
		HORMONE-TREATED†	UNTREATED
Lantana	4.0	25.9	3.4
	5.0	35.7	3.8
	7.0	36.5	24.5
Acalypha	1.0	57.5	9.3
Fig	2.0	1.1	0.0
	3.0	8.9	5.7
	3.5	11.0	6.1
Tradescantia	2.0	4.0	0.0
(internodes)	2.5	6.3	0.0

* *Tradescantia* cuttings were placed in tap water, all others in sand.

† All treated and untreated lots consisted of 10 cuttings in all instances except *Acalypha*, which consisted of only 6 cuttings.

As with the lemon, *Lantana*, and *Acalypha*, the hormone-treated fig cuttings showed increased rooting as compared with controls.

Experiments with *Tradescantia* were made with internode cuttings without nodes and leaves. These, when placed in tap water and treated with hormone, formed roots within two weeks on the four internodes which were originally nearest the tips of the vine from which the cuttings were made, but did not form roots on the internodes which had been farther from the tip. No roots were formed on any of the untreated internodes.

These results show that treating with β-indolyl-acetic acid not only increased rooting with cuttings that will root when untreated, but also caused the formation of roots on leafless lemon and *Tradescantia* cuttings which do not ordinarily form roots. Other experiments are now in progress to determine the effect of this and other hormones on cuttings of other plants with particular attention to cuttings from plants which are difficult to root, such as the apple.

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THE STOMATA AND TRANSPIRATION OF OAKS

L. Edwin Yocum

(WITH TWO FIGURES)

Introductory

Stomata and transpiration are of fundamental importance in the water relations of plants. It is evident that the loss of moisture in transpiration is equally as important as absorption of water by the root system in plant survival. Our mesophytic plants must have stomatal protection against excessive loss of moisture during drought. This is of paramount importance to us in our conservation work, where trees are being used as windbreaks, and to conserve moisture. Most of our knowledge regarding stomata has to do with herbaceous plants, as indicated by texts in plant physiology (Miller, **7**, and others). Growth habits and therefore soil conditions are not the same for leaf formation on herbaceous plants as on trees. On trees most of the leaves grow simultaneously in the spring while on herbaceous plants they appear over a longer period. In herbaceous plants stomata are commonly on both leaf surfaces, but tree leaves have them usually only on the lower surface.

Nearly all the water loss of plants occurs through the stomata. Some is lost through the epidermis, and since the upper epidermis has thicker cell walls, it is probable that a little more water loss occurs through the lower than through the upper epidermis. Water passes freely through the stomata when open; in fact Brown and Escombe (**2**) have shown that water may be lost through the stomata as rapidly as though it were evaporating from a free water surface instead of through many small pores. Sayre (**12**) has shown that the rate of transpiration depends more on the perimeter of the opening, than on its area, which is very important in the study of partly closed stomata. It is evident from their shape that the beginning of closure changes the perimeter very little, and only the last few per cent. of closure will in many cases affect the transpiration rate, as found by Jeffreys (**4**).

Methods

The relation of the stomata to transpiration was measured by the cobalt chloride paper method, since it seemed to offer a more accurate measure than the actual observational measurement of the final stages of closure in percentages. Mature leaves on one to three year old *Q. palustris*, *Q. rubra*, and *Q. prinus* seedlings grown outdoors, some in pots and some in seed beds, were used. Readings were taken at intervals of two hours or less over twenty-four hour periods.

Stomatal counts were made on fresh leaves of the above seedlings and from mature leaves from trees with the aid of the "ultropak" microscope at 500× magnification. REED (10) has shown that immature leaves of citrus have many more stomata per mm.^{2} than mature ones. At least ten leaves were used from two or more trees of each species. The trees were isolated individuals or at the edge of a group where they were freely exposed to light and wind. To select trees within a group would reduce the number of stomata, as SALISBURY (11) found the number to decrease with the protection of the forest.

The stomata were counted in a rectangle 200 μ by 150 μ, except in the case of the willow oak at 10 places on each leaf, or a total area of 0.3 mm.^{2} per leaf. Areas having veins thirty μ or more in width were avoided. In order to get the average number of stomata, five counts were made on each side of the midrib as follows: near the base of the leaf; near the midrib at the center of the leaf; near the center of the halfleaf; the edge opposite this point; and near the tip. Counts were made on willow oak leaves only at the base, middle, and tip.

Results

During the day, with adequate soil moisture the cobalt chloride paper reacted over the lower epidermis in one twentieth to one tenth the time required over the upper epidermis. At sunset the reaction time of the lower epidermis quickly changed to about one half the time on the upper side, but at sunrise it returned to daytime rates. Seedlings[1] grown in a seedbed in 1931 during a very dry period, when the soil moisture was below the wilting coefficient near the surface and a little above the wilting coefficient deeper in the soil, were found to have their stomata closed all day as indicated by their transpiration. The cobalt chloride paper required two hours or longer to change color on the lower surface and usually a little longer on the upper, due to the difference in epidermal cell walls. If they were entirely closed it was thought that carbon dioxide would be prevented from entering and that photosynthesis would be retarded. Leaves tested with iodine for starch at 4:00 P. M. on bright days gave a negative test for starch. A second seedbed was watered artificially and showed a high rate of transpiration and an abundance of starch in the leaves. Two days after a heavy rainfall the seedlings in the dry bed showed rapid transpiration and photosynthesis. A negative test for starch on a day favorable for photosynthesis appears to be a good test for all day total stomatal closure.

The differences in stomatal numbers for various parts of the same leaf were small, but the base of the leaves always had the fewest stomata; and in nearly all cases the region near the midrib had a lower number than the

[1] Results of studies made while employed by the Allegheny Forest Experiment Station, Philadelphia, Pa.

other parts of the leaf. No consistent differences were noted in the middle, edge, and tip counts, but in the total count the edge and tip were nearly 0.25 per cent. greater than the middle; the midrib, nearly 2, and the base, nearly 8 per cent. less. That is, in a general way the smallest number was found at the leaf base, and it increased toward the tip and edges of the leaf. Shaded leaves on mature trees and on seedlings always had fewer stomata than insolated leaves.

Stomata are small but very numerous and the guard cells may cover more than fifty per cent. of the surface as is shown by *Q. triloba* (fig. 1), and forty per cent. in the case of *Q. palustris* (fig. 2). Small areas of *Q. triloba*

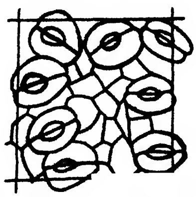

FIG. 1. *Q. triloba* Michx., stomata and epidermal cells in an area of 0.0036 mm.^{2}

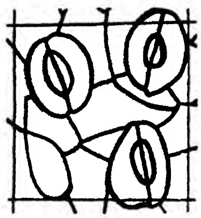

FIG. 2. *Q. palustris* DuRoi, stomata and epidermal cells in an area of 0.0036 mm.^{2}

have an average of more than nineteen hundred per mm.^{2}, and the average for all counts was 1192 per mm.^{2} This is exceeded to the writer's knowledge only by ADAMSON's (1) report of *Veronica cookiana,* 2200. Other species of *Veronica* had an average number of 250 to 400 per mm.^{2} Fully opened stomata of *Q. triloba* are about five μ long and one μ wide, or about

TABLE I

STOMATAL NUMBERS FOR *QUERCUS* SP. PER MM.^{2}

TREES

PLUSTRIS DUROI PIN	*VELUTINA* LAM. BLACK	*RUBRA* L. RED	*STELLATA* WANG. POST	*PHELLOS* L. WILLOW	*PRINUS* L. CHESTNUT	*COCCINEA* WANG. SCARLET	*TRILOBA* MICHX. SPANISH
497	580	680	693	723	890	1038	1192
SEEDLINGS							
539		836	635				

one per cent. of the total surface of the leaf. Table I gives the average stomatal numbers for trees of eight species, and three seedlings.

Stomatal counts were made at different elevations on two species of oaks, *Q. palustris* at 7, 17, and 60 feet, and *Q. velutina* at 7, 14, and 30 feet, taken from all sides of exposed trees. The results were similar for the two species. The data given for *Q. palustris* in table II are the average of 100 or more

TABLE II

STOMATAL NUMBERS FOR A *QUERCUS PALUSTRIS* DUROI TREE AT THREE ELEVATIONS

HEIGHT ON TREE	AVERAGE NUMBER PER 0.03 MM.^{2} FOR EACH REGION OF LEAF						
	MIDDLE	EDGE	TIP	MIDRIB	BASE	TOTALS	PER MM.^{2}
ft.							
7	15.85	15.85	15.57	15.36	14.21	2154	511
17	14.50	14.20	14.70	14.65	13.65	1434	481
60	15.40	14.85	15.30	14.60	14.00	1482	494
Average	15.31	15.08	15.25	14.88	13.96		496

counts for each elevation. The number per mm.^{2} for each elevation is within three per cent. of the average number and while at 7 feet high the averages are higher, it is probably not significant.

In order to examine further the relation of elevation to stomatal number the shoot of a three year old *Q. palustris* seedling was removed at the ground level in the winter, and a single sprout allowed to grow the following spring. On July 19, the sprout was forty-four inches tall. Stomatal counts were made at eight inch intervals from the ground as follows:

Height from the ground, inches	8	16	24	32	40
Stomata per mm.^{2} . .	410	430	450	503	533

The leaves at the forty inch level were mature and had developed during July. This shoot had developed its leaves progressively as is common for herbaceous plants.

Discussion

The transpiration rate of the oaks studied showed that the stomata normally remain open throughout the day but close at sunset. With low soil moisture the stomata may remain closed all day even to the extent of preventing photosynthesis. This action of the stomata is very important as it indicates a maximum transpiration rate when soil water is available, justifying the statement sometimes made that "trees are our natural humidifiers"; and when soil water is so limited that many herbaceous plants would be seriously injured or killed, trees may resist drought for some time

because of the action of their stomata. Thus in addition to improving the physical conditions of the soil for the retention of soil moisture the oaks give off water rapidly when it is abundant in the soil but withhold it during drought. MAGNESS (5) has reported similar drought reactions for apple trees in the eastern states, and finds that there is a cessation of growth of fruit, but that the stomata rarely remain open later than noon on bright clear days, regardless of the soil moisture conditions.

This difference in afternoon stomatal condition suggests interesting questions regarding the rate of photosynthesis in apples and oaks. Is the rate much higher for apples in the morning than for oaks, or is the total amount greater for oaks? Of equal interest and perhaps more important to conservationists is the "water requirement" of trees, or as MAXIMOV (6) prefers to state it, the "efficiency of transpiration." Are we more interested in the maximum humidifying power of the plant or in the maximum plant material produced with the available water? SCARTH (13) has discussed the problem of the stomatal influence on transpiration and photosynthesis.

The stomatal counts for the species recorded were made during the summer of 1934. During the previous six months, December to May, inclusive, the total rainfall was greater than normal by more than six inches; during only two months, January and April, was it slightly below normal, according to the U. S. Weather Bureau records for Washington. Excessive rainfall during the spring tends to decrease the stomatal numbers, according to HIRANO (3); therefore the frequencies given in table I are lower than the number to be found during a dryer spring. The number of stomata found on trees growing in a dense forest would probably be lower because of the high percentage of shaded leaves, which were found to have fewer stomata. HIRANO (3) points out some ecological relationships to stomatal numbers in citrus. The oak species examined in this study have not indicated a well defined ecological relationship; however, the three species having the greatest frequency grow on dry soil.

Trees generally have a higher stomatal frequency than shrubs or herbaceous plants as found by SALISBURY (11), RABER (9), and others. Citrus has been studied extensively by HIRANO (3), OPPENHEIM (8), and REED (10), who found a frequency often more than 500 per mm.^{2} and a distribution similar to that found in oaks. No record has been noted of a group of species with as high stomatal frequencies as are found here in the oaks.

SALISBURY (11), YAPP (14), and others have found a rapid increase in the stomatal numbers as the leaves are higher on the stem. REED (10) finds a certain relationship on long stems of citrus but not on short ones. MAXIMOV (6) describes YAPP's work under the heading "Leaf structure in relation to water," but follows the suggestion of ZALENSKI that the later leaves

have more water deflected to the lower leaves, causing a greater osmotic pressure in the upper leaves, producing more stomata. It has been shown by HIRANO (3) that the amount of spring rainfall has more influence on stomatal number than has annual rainfall. Herbaceous plants grow their leaves during several months. In YAPP'S (14) study of *Spiraea*, illustrations are given from March to June, inclusive, during which time the external conditions favor the increase of transpiration as new leaves develop, but normally the soil moisture becomes less. This would cause the osmotic pressure of the leaf cells to become greater and the leaves to become more xeromorphic, with increased stomata. The difference in height would have relatively no effect compared with the difference in the resistance which the soil offers to water loss in early spring and in the dryer summer period. This would seem to explain the fact that stomatal frequency varies so little on the *Q. palustris* tree with a height of sixty feet since the leaves all developed with nearly the same soil moisture; but a seedling of the same species has thirty per cent. more stomata at forty inches than it has thirty-two inches lower on the same stem.

Summary

1. The stomata of oaks are functional and may remain completely closed during a drought.

2. Stomatal frequencies on eight species of oaks are all high, and on *Q. triloba*, the highest, the frequency is exceptionally high.

3. Stomatal numbers do not appear to increase with height on mature, exposed trees, but they do appear to increase on indefinitely growing stems.

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PHOTOSYNTHESIS IN *GRIMMIA MONTANA*[1]

ELIZABETH McKAY

(WITH ONE FIGURE)

Introduction

The xerophytic moss, *Grimmia montana,* is known commonly as "black moss." During the greater part of the year the name is fully justified, for when dry the moss appears on the dark basaltic rock outcrops of southern Washington as a small black cushion 1–6 cm. wide, composed of a dense tuft of plants from 1 to 2 cm. high. The tuft usually shows slightly gray, owing to the presence of long hyaline points on the leaves. In addition to the long transparent points, *G. montana* shows a number of other xeromorphic characters (**13**). The margins of the leaves are revolute and the leaves are slender and pointed, with small, thick walled cells. The plants grow in dense cushions, reducing the surface exposed to insolation.

When the moss is dampened the changes produced are immediate and marked. The leaves unfold, the plant appears to expand, and the dull gray-black color is replaced by a vivid green. Apparently this change in appearance is followed shortly by a decided activation of life processes in the plant.

The object of this investigation was to attempt to determine the time elapsing after the addition of moisture to the air-dry plant before photosynthetic activity begins.

Literature

FRAYMOUTH (**3**) quotes BASTIT (1891) and JONSSON (1894) as having found that dry mosses respire and that respiration increases with increase of the water content.

IRMSCHER (**6**) concludes that the drought resistance and the marked changes of a given species of moss in different habitats are due to the great adaptability of the protoplasts to changes in environment. This characteristic is not great enough for the survival of rapid alternate desiccation and moistening. In addition to the peculiarity of the protoplasts, the mosses possess dormant buds which survive more severe conditions than the ordinary cells and develop new protonema when conditions are favorable.

DAVY DE VIRVILLE (**1, 2**) has grown several species of moss and has noted the effects of light, temperature, and humidity on the structure of

[1] Contribution no. 40 from the Botany Department of the State College of Washington.

the plants. His conclusion is that, even though a moss is normally xerophytic, high humidity is more favorable to its growth.

Mayer and Plantefol (**9, 10, 12**) find that mosses retain some water even at 100° C. In *Hypnum triquetrum* this amounts to 5–6 per cent. They find also that a moss with water content of 8 per cent. will absorb water to 18 per cent. of its weight when placed in a saturated solution of NaCl. From these data they conclude that the force involved in resisting desiccation is imbibitional rather than osmotic. Photosynthetic activity of *H. triquetrum* increases with increase of water content up to 300 per cent. Respiration increases up to 150 per cent. Decrease in the water content causes a more rapid decrease in photosynthesis than in respiration, so that apparently respiration is less directly dependent upon the water content than is photosynthesis.

By estimation of the oxygen absorbed, Fraymouth (**3**) has correlated the increase of respiration with increase of water in several lower plant forms, including algae, lichens, and the moss *Hypnum cupressiforme.* She finds that respiration in *H. cupressiforme* increases with increase of water content up to 300 per cent. of water.

Iljin (**5**) has studied numerous vegetative cells, both resistant and non-resistant to desiccation, and concludes that the hardening of a plant is a process of devacuolization. The ability of a cell to withstand desiccation is due to lack of vacuoles, and the reason the Grimmiaceae are resistant is that they lack these structures.

Material and methods

First method.—Two methods were used in determining the beginning of photosynthetic activity in *Grimmia montana.* In the first method the moss was immersed in water and the change of resistance of the water was measured with the submerged moss exposed to bright sunlight. Redistilled water was employed, but the purity of this water did not approach that necessary for exact quantitative tests, as is shown by variation in the measurements from 10,125 to 12,375 ohms. The water was kept in the laboratory from a few hours to several days. During this time it was stored in glass flasks with cotton stoppers so that the atmospheric carbon dioxide could dissolve freely. A second measurement was made duplicating the conditions except that the moss was placed in darkness. It was believed that the plant placed in darkness would show, owing to respiration, a gradual increase in the carbon dioxide evolved and hence in the conductivity of the solution in which it was submerged. The plant which was exposed to sunlight, and was therefore free to carry on photosynthesis, would utilize some of the carbon dioxide produced and show either a smaller decrease of resistance than that shown by material kept in darkness

or an actual increase of the resistance. The length of time elapsing before the darkened and illuminated samples showed a difference of resistance was taken as a measure of the time necessary for the beginning of photosynthesis. In order to check the possibility of resistance changes due to solution of carbon dioxide from the atmosphere of the greenhouse, tests were run on the distilled water without moss, through similar periods. These samples showed no measurable changes of resistance.

Material for the resistance tests was collected during the summer season. At that time the moss had received very little moisture for several weeks and was practically air-dry. As nearly as possible the material was collected from places exposed to direct sunlight during either the morning or afternoon, and those tufts were chosen which appeared to have the same degree of hyaline leaf point development. In the laboratory the tops were cut from the plants, leaving the rhizoids and as much as possible of the earth in which they were imbedded as waste material. The material that remained, after trimming, included the tops of the stems with the living leaves.

The leafy stems were then washed and rinsed several times with distilled water in order to remove all possible material which might contain electrolytes and cause resistance changes. After being washed, the tops were air-dried and weighed in samples of 1 gm. each. Before weighing, any remaining foreign material was carefully removed from the sample. Between the time of preparation and the time at which the moss was used, it was stored in glass bottles. In determining the water content, samples were dried, in accordance with the recommendations of LINK and TOTTINGHAM (**8**), at 65° C. to a constant weight. The loss was found to be 0.2 gm. from a sample weighing 4 gm., or 5 per cent. of the air-dry weight after 42 hours' drying.

The sample, after being washed and dried, was placed in a measured quantity of distilled water in a 250-cc. beaker and kept at a temperature of 24° ± 0.5°. Using a wheatstone bridge, measurements were made of the resistance changes in the water at short intervals for about two hours following immersion of the material. The moss was stirred frequently, to expose all parts to the sunlight and to distribute the CO_2 uniformly throughout the medium. In the same manner, samples were placed in water and the change of resistance was measured, except that the beaker was surrounded by several thicknesses of black cloth, to exclude the possibility of photosynthesis. In this case also the solution was stirred frequently and the temperature maintained at 24° C. ± 0.5°. The results obtained by these resistance measurements are only qualitative, because of several uncontrolled factors. The intensity of the sunlight varied with the different determinations, and, owing to the occasional passage of a

cloud, during each determination. The original purity of the water varied somewhat and the nature of the impurities was not ascertained. This offers opportunity for change of resistance resulting from reactions of the carbon dioxide, such as the formation of ammonium carbonate, rather than directly from the carbon dioxide changes. A third possibility of error lies in the leaching of electrolytes and buffer solutions from the cells of the plant, and the dissolving of material not removed from the exterior of the plant by washing. The solution of carbon dioxide from the atmosphere was shown by resistance measurements of the distilled water to be insufficient to produce any measurable change in resistance.

SECOND METHOD.—In this method measurement of the increase in reducing sugars was used as an indicator. The moss was collected during the period when moisture was relatively abundant and the plant was growing. The moss was placed in darkness, while damp, and allowed to dry slowly to air-dryness. Preliminary comparison of the sugar content of samples dried more rapidly in light with those dried in darkness showed that, in the latter, the excess sugar was to a large extent used by the plant while photosynthesis was not being carried on. The samples were allowed to remain in the air-dry state for several days before tests were made. A small piece of the moss cushion was then selected and a microchemical sugar test was made on the dry material, by placing a few leaves on a glass slide, adding a few drops of Fehling's solution of HAWK's formula (4), and warming. The leaves were observed under a microscope and the amount of precipitate formed at definite time intervals was noted.

After a few dry leaves had been tested, the piece of moss was dampened well and placed in bright sunlight, in a watchglass containing a small amount of tap water. Sugar tests were made and the relative amounts of precipitate were recorded. It was found that leaves of the stem tip bearing archegonia contained more sugar than those of a vegetative stem tip under the same conditions, so care was used in the tests to obtain leaves of the same kind for all tests of a single sample.

Results and conclusions

The results obtained by the measurement of the change of resistance are shown in figure 1, which is a representative curve chosen from a number of determinations. Measurements were made using 1-gm. samples of dry moss in 225 cc. of water at a temperature of 24° C. The solid line shows the change of resistance of the solution surrounding a moss sample when the moss is exposed to sunlight; the dotted line shows the change when the moss is kept in darkness.

The first 10 minutes show an abrupt decrease in resistance, apparently due to the solution of material not removed from the moss in washing or

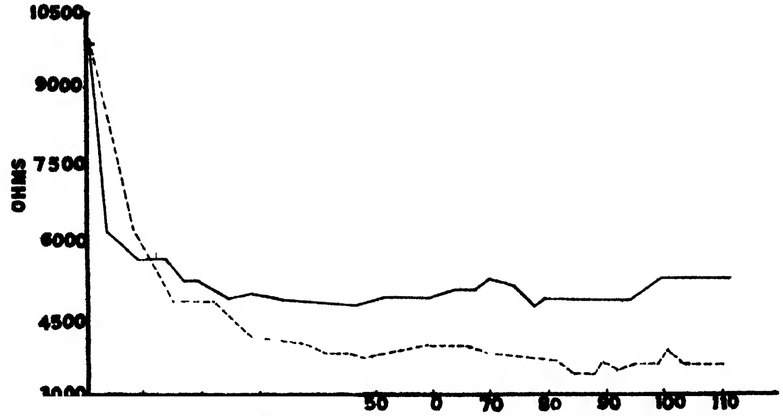

FIG. 1. Changes in resistance of the medium during photosynthesis and respiration of *Grimmia montana*.

to the leaching of cell contents. In the period from 10 to 20 minutes the curves flatten and differences in resistance begin to show between the two. The curves show minor fluctuations, but the darkened sample shows a gradual lowering, expressing the increase of carbon dioxide from respiration in the absence of photosynthesis. The curve for the illuminated sample reaches a level between 30 and 40 minutes, showing that the photosynthetic reaction has apparently reached an equilibrium with the respiratory process and atmospheric absorption, resulting in a nearly constant carbon dioxide supply.

The conclusion is reached that photosynthesis begins in *Grimmia montana* from 10 to 20 minutes after the plant is immersed in water and illuminated, and that within 25 minutes the activity is great enough to utilize the carbon dioxide produced in respiration as well as that supplied by the atmosphere. Within 40 minutes the plant apparently reaches a photosynthetic equilibrium for the given temperature and light conditions.

The quantities of reducing sugars in the moss after receiving moisture and light show no quantitative change in the cells of the leaf within 5 minutes. Within 7 or 8 minutes a slight difference is shown. After 10 minutes the increase is decided, and after 25 minutes, fairly large. At the end of 30 minutes there is no further increase, indicating that the process has reached an equilibrium, possibly because of the accumulation of photosynthetic products.

The microchemical tests show a somewhat shorter period before photosynthesis begins than is shown by the resistance measurements. This is probably explained by the slight buffer action of the carbonic acid, which would retard the change of resistance so that the time found in the comparison of

resistance changes in water surrounding illuminated and darkened moss may be somewhat longer than is actually required for photosynthesis to begin.

Summary

1. The time required after moisture is added to a xerophytic moss, *Grimmia montana,* before photosynthesis begins, was determined.

2. The air-dry moss, when heated at 65° C. to a constant weight, was found to have lost 5 per cent. of the air-dry weight.

3. Measurements were made of the change of resistance of water surrounding the moss in samples that were illuminated and samples that were kept dark. The change of resistance was assumed to be due to changes in the amount of carbon dioxide, resulting either from respiratory or from photosynthetic activity.

4. Microchemical tests were made using Fehling's solution to determine the change in amount of reducing sugars in the moss after being dampened, as a measure of the time required for photosynthesis to begin.

5. Photosynthesis apparently begins 6 to 10 minutes after water is supplied to the moss, is great enough within 25 minutes to utilize the carbon dioxide produced by respiration, and reaches an equilibrium within 30 to 40 minutes.

The writer wishes to thank Dr. F. L. Pickett for suggestion of the problem and helpful criticism of the work.

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SOME CHEMICAL ASPECTS OF CALCIUM DEFICIENCY EFFECTS ON *PISUM SATIVUM*

DOROTHY DAY

The beneficial effect of calcium in the growing of crops has been known through the ages but it is only in modern times that any detailed investigations have been made to see just how this element affects the individual plant or its parts. Various criteria have been used, usually based on the external appearance of the plant, but more recently attention has been turned to the effect on the internal anatomy caused by lack of this element, as in the work of SOROKIN and SOMMER on the pea plant (5). WARINGTON (6) has approached the problem from the chemical point of view with quantitative estimations of the amount of calcium absorbed by *Vicia faba* at different stages of growth and under varying nutritional conditions.

It has been the object throughout this research to combine and correlate the anatomical aspect with the chemical. This was begun by growing all of the plants two in a pot so that both received exactly the same treatment. Thus it was possible to examine the internal structure of one plant and to use the other for determinations of weight and of calcium content. A preliminary report summarized all results (**1**). Surprizingly enough, an anatomical examination (**2**) of plants treated with various solutions showed almost no variation in the internal structure of root and stem even when calcium was completely withheld, and the chief difference between plants so unlike in size and succulence was in the amount of elongation of their several organs.

Methods

Canada field peas (*Pisum sativum* L.) were grown in sand cultures in the greenhouse under controlled conditions. Details of the methods and materials have been given previously (**2**). Seven kinds of nutrient solutions were used to meet the mineral requirements of the plants, six pots to a treatment. These solutions can be grouped in three categories according to their composition and also according to their physiological effect: A, those with an optimum of calcium; B, D, F, those with one-half that amount; and C, E, G, those with no calcium. Solution A is the one that preliminary experiments had shown was best suited to the growth of this species (**2**). In solutions A, B, and C the only difference is in the amount of calcium nitrate; solution B contains one-half the quantity present in A, while C lacks it entirely. In solutions D and E this same ratio holds but the nitrate content is compensated in chemically equivalent amounts by equal parts of sodium nitrate and potassium nitrate. In solutions F and G

there is a similar substitution for the nitrate, but one-fourth of the nitrate is replaced by magnesium nitrate and three-fourths by potassium nitrate; therefore there is a substitution for the nitrate radical rather than a molecular substitution.

All plants were harvested at the end of a five weeks' period of growth, just before the death of the plants grown without calcium, although those given one-half the full amount of this element were still fairly vigorous and probably could have lived for some time longer. From each pot one plant was fixed for anatomical observations and the other was used for fresh weight and dry weight determinations of shoots and of roots. After drying, this material was pulverized preliminary to the quantitative analysis for calcium, which was performed with duplicate samples according to McCrudden's method (**4**). Results were recorded in terms of calcium oxide.

Results

In general, the plants of the complete nutrient solution (A) seemed to be fairly uniform in height with an average of twenty-eight inches above the cotyledonary node. Those plants given the solutions with one-half the amount of calcium (B, D, F) form a fairly uniform group and were almost as tall as those with the complete nutrient solution (A). There was, however, a decided difference between any of these and the plants deprived of calcium (C, E, G), which formed a third distinct group; these were about one-half as tall as those given the complete nutrient solution (A).

The shoots of those plants receiving the full ration of calcium (A) showed the greatest average fresh weight, while those given one-half the amount of calcium (B, D, F) were almost as heavy. However, those starved of calcium (C, E, G) were less than one-third as heavy as the plants treated with the solution containing the one-half ration of calcium. Roots of these same plants showed similar relationships except that there was not quite so decided a difference between the plants starved of calcium and those given the half amount of this element although it was still marked. Details of these weights are given in table I.

The greatest average dry weight of shoots was shown by the plants receiving the complete nutrient solution (A). Those given some calcium (B, D, F) were slightly lighter, while those deprived of this element (C, E, G) were definitely the lightest, although the differences were not so marked in any case as those noted for the fresh weights. Roots of these same plants showed similar results except that the difference is even less between the plants starved of calcium and those given some. The data are included in table I.

The percentage of dry matter in the shoots is greatest in the plants starved of calcium and least in the plants given the most calcium. The

TABLE I

FRESH WEIGHT, DRY WEIGHT, AND CALCIUM CONTENT OF CANADA FIELD PEAS (*PISUM SATIVUM* L.)

NUTRIENT SOLUTIONS	COMPLETE	ONE-HALF CALCIUM			NO CALCIUM		
	A	B	D	F	C	E	G
		$Ca(NO_3)_2$ UNREPLACED	$Ca(NO_3)_2$ REPLACED BY EQUAL PARTS OF $NaNO_3$ AND KNO_3	$Ca(NO_3)_2$ REPLACED BY NITRATE MIXTURE $\frac{1}{4}$ $Mg(NO_3)_2$ AND $\frac{3}{4}$ KNO_3	$Ca(NO_3)_2$ UNREPLACED	$Ca(NO_3)_2$ REPLACED BY EQUAL PARTS OF $NaNO_3$ AND KNO_3	$Ca(NO_3)_2$ REPLACED BY NITRATE MIXTURE $\frac{1}{4}$ $Mg(NO_3)_2$ AND $\frac{3}{4}$ KNO_3
SHOOTS							
Av. fresh wt. gm.	12.163	10.090	11.003	10.684	3.371	3.276	3.104
Av. dry wt., gm.	1.339	1.213	1.295	1.234	0.506	0.480	0.425
Per cent. of dry matter	11.009	12.022	11.770	11.550	15.010	14.652	13.692
Av. CaO per plant, gm.	0.028	0.023	0.019	0.023	0.010	0.009	0.009
CaO, per cent. of dry wt.	2.125	1.900	1.500	1.825	2.000	1.850	2.200
CaO, per cent. of fresh wt.	0.230	0.228	0.173	0.206	0.297	0.275	0.290
ROOTS							
Av. fresh wt. gm.	6.629	4.735	5.933	5.601	2.093	2.611	2.371
Av. dry wt., gm.	0.591	0.372	0.515	0.496	0.165	0.204	0.170
Per cent. of dry matter	8.915	7.856	8.680	8.856	7.883	7.813	7.170
Av. CaO per plant, gm.	0.013	0.009	0.013	0.008	0.004	0.004	0.006
CaO, per cent. of dry wt.	2.250	2.500	2.500	1.550	2.665	1.940	3.330
CaO, per cent. of fresh wt.	0.196	0.190	0.219	0.143	0.191	0.153	0.253

contrary is true for the roots but the difference is slight. The exact proportion of dry weight to fresh weight in each case is shown in table I.

The plants given the most calcium in the nutrient solution contained the most at the end of the growth period of five weeks. For both shoots and roots the plants treated with the half ration showed three-fourths as much calcium as those plants offered the complete nutrient solution. Those deprived of this element had almost one-third as much calcium as those with the complete nutrient solution. In general, the plants having equal amounts of calcium in the various nutrient solutions were similar. However, decidedly less calcium was present in the shoots of solution D (one-half calcium) and in the shoots and roots of solution E (no calcium). These were the solutions in which the calcium nitrate of the complete nutrient solution was replaced by equal parts of sodium nitrate and potassium nitrate.

Calcium oxide, computed as percentage of the dry weight of the plant, is generally greater in the roots than it is in the shoots. It is greatest for the roots of the plants deprived of calcium and least for the shoots of plants given the one-half amount of calcium. It is lower in shoots from solution D and in plants from solution E as has been noted for the entire plant.

Calcium oxide, calculated as percentage of the fresh weight of the plant, is higher for the shoots, in general, than it is for the roots. This is the reverse of the relation just noted in which the roots show more calcium as percentage of dry weight. The percentage of fresh weight is greatest for the shoots of plants starved of calcium while the roots of these plants show almost no difference from the plants given the complete nutrient solution.

Discussion

An optimum of calcium increases both the number of internodes and the length of the internodes, and increases the fresh and dry weight in comparison with plants given only one-half as much calcium. In all these respects the difference is even more striking between fully nourished plants and those deprived of calcium.

Calculations of the dry weight in relation to the fresh weight show that, for the quantities of calcium used here, the roots have a slightly greater proportion of dry weight according to the amount of calcium present in the nutrient solution. Probably there is some storage of material without much leaching in the presence of calcium. For the shoots the greater the amount of calcium in the nutrient solution the less is the percentage of dry matter and the greater is that of water present. This appears to indicate that calcium is conducive to succulence. However, other workers have ascribed this same result to other elements, especially nitrogen, used in similar experiments. With the possible exception of nitrogen, might it be said that

succulence should be associated with good nutrition in general rather than with the presence of any particular substance? This seems to be the contention of FAMIN (3), who disagreed with VESQUE, VAN RYSSELBERGHE, and PETRIE since he found that the majority of salts which he studied do not increase the succulence of young and vigorous tissues.

Since most calcium was present in the complete nutrient solution it is not surprising that the roots and stems grown there should show the most calcium oxide per plant. The close approach made to this by the plants given only one-half that amount of calcium may indicate that a plant uses most of the material stored in the seed, that it makes the most of any calcium supplied, that it needs little calcium, or all three. The relatively large amounts of calcium oxide present in the plants grown in the solutions deficient in calcium may support one or more of these points. It must be remembered that all plants were harvested just before the death of these last groups of plants although the partially starved plants were still fairly vigorous and would have lived for some time longer. WARINGTON (6), working with *Vicia faba,* also found that the amount of calcium absorbed is proportional to the calcium supplied in the nutrient solution.

Summary

1. Canada field peas were grown under greenhouse conditions in nutrient solutions in which the proportion of calcium nitrate was varied. External appearance, fresh weight, and dry weight were observed and measured. Samples were analyzed for calcium content according to MCCRUDDEN'S method.

2. These results support the contention that lack of calcium affects the external appearance of pea plants, making them shorter and less succulent.

3. Both shoots and roots of the plants given the complete nutrient solution showed the greatest fresh and dry weights; those given a half ration of calcium were almost as heavy, and those deprived of calcium were decidedly lighter.

4. The percentage of dry matter in the shoots is greatest in the plants deprived of calcium. The contrary holds true for the roots but the difference is slight.

5. The plants given the most calcium in the nutrient solution contained the most at the end of the growth period of five weeks. Those starved of calcium had almost one-third as much calcium as those grown in the complete nutrient solution.

6. There was less calcium in the plants in which the calcium nitrate of the complete nutrient solution was replaced by equal parts of sodium nitrate and potassium nitrate than in those grown in solutions in which the calcium nitrate was not compensated or was replaced by magnesium nitrate and potassium nitrate.

7. There is no consistent variation in the proportion of stored calcium to fresh weight or to dry weight.

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SOME EFFECTS OF FUEL OIL ON PLANTS

George D. Fuller and Margaret R. Leadbeater

Some years ago a storage tank of fuel oil emptied upon the soil is supposed to have caused the death of several oak trees in a suburb of Chicago. This caused the writers to make certain preliminary experiments on the action of such oil on certain potted plants.

While attention had been directed towards the entrance of such oils into the leaves of plants when applied as a spray (see among others Ginsberg (2), Knight *et al.* (4), Kelley (3), de Ong (1), and Rohrbaugh (5)), no investigation regarding their effects upon roots is known to the writers.

The fuel oil used was purchased from the Consumers Petroleum Company, Chicago, Illinois. It is described commercially as 3236 Baume gravity gas oil, consisting principally of saturated hydrocarbons. It is obtained in the refining of petroleum as one of the heavier fractions of distillation. The viscosity is about 55 seconds Saybolt. In appearance it is medium thin, of a greenish amber color, and is similar to the "medium oils" which spray investigators have found most useful.

The experiments were carried on with four plants: the tomato, *Lycopersicum esculentum;* the peach, *Prunus persica;* the apple, *Pyrus malus;* and *Ageratum houstonianum.* The four were chosen for their availability rather than for any scientific reason, but they proved suitable material for the investigation because none of them contained natural oil in sufficient quantities to interfere with the recognition of the introduced oil in the plant body, and they gave an interesting variety of reactions. As the most extensive experiments were with tomatoes and peaches, the reactions of these two species only are being reported.

The tomatoes were from greenhouse stock, all from the same planting, carbohydrate high and tending to reproduction. The peaches and apples were year-old seedlings obtained through the courtesy of Dr. M. J. Dorsey, Chief in Pomology at the University of Illinois. They were planted in good potting loam, the tomatoes being in 5-inch pots and the peaches in 6-inch. Two lots of 12 plants each were used for each species divided into sets of two pots each. One of these sets, without oil, served as the control; the other five sets had one application of oil applied to the surface of the soil in amounts of 1, 2, 3, 4, and 5 per cent. by volume of the soil content of the respective pots. In one lot of each species the soil was heaped about the stems of the plant so that there was no surface contact of the oil with the stems. This was designated as the *M* series; the others constituted the *S* series. All plants were watered regularly to maintain proper moisture conditions for good growth and the plants were kept under observation for 51 days.

Results with tomatoes

The control plants and part of those in soil with 1 per cent. of oil appeared quite healthy and vigorous at the end of the period. The plants of the *M* series usually showed injury a day or two before those of the *S* series.

The first signs of injury were the wilting of the youngest two or three leaves of the main axis and those of the lateral axes. The whole tip sometimes wilted with the young leaves. Leaf wilting occurred with or without discoloration. In case of discoloration, the leaves turned either olive-green or yellow. No oil was found in leaves which had turned olive although it looked from the outside as if it might be present. Flowers developed if the buds were fairly mature when injury became apparent, otherwise they blighted.

Yellowing of the older leaves was not taken as a symptom because the plants stood rather close together and the checks dropped their oldest leaves also. However, as the injury advanced all the leaves of the plants yellowed, wilted, and died. Sometimes the stem collapsed near the surface of the ground or halfway up the stem, sometimes it remained upright. This seemed to be a matter of individual resistance for there was no correlation between doses of oil and methods of degeneration. Wilting occurred in from eight to fourteen days for the larger doses and twelve to twenty for the smaller ones. There was less difference in the time of death of the two groups. It occurred in twenty-two to twenty-eight days in the plants in soil with 4 to 5 per cent. of oil, in twenty-four to forty-two days for those with 3 per cent. or less, and half the plants in soil with 2 per cent. of oil were still alive at the end of the experiment.

Sections were made with the use of the freezing microtome from plants of both the *M* and *S* series grown in soil with 1, 3, and 5 per cent. of oil. These sections were taken from the tips of the plants, from the mid-stems, from the stems at the surface of the soil, and from the roots. They were stained with Sudan III and examined for the presence of oil, which was found in varying amounts in all the plants, with larger amounts in plants from soil with highest oil content.

The oil was found most regularly in the xylem, particularly the primary, where the stain brought out small drops clinging to the sides of the vessels or completely clogging them. Only where it was plentiful did it spread to the xylem parenchyma or to the rays, being confined to the bundles except when present in excess. It was next most prominent in the intercellular spaces of the pith and cortex. In the root, it was about equally distributed between xylem and cortex, but it was often difficult to tell about the location because degeneration had proceeded so far that the structures were but ill defined. Judging by color and texture, the roots were certainly badly injured by the oil, although they did not always show great quantities of it within them. Roots of check or low-quantity treatment plants were cream

to light tan in color, firm, and brittle. Those severely affected by higher percentages were dark red-brown to black, withered, and limp.

Results with peaches

Almost a month elapsed before the peaches showed any response to the oil treatment. A very small plant (*M* 5 per cent.) was wilted when visited on the twenty-fifth day. Very soon afterwards all but one of those at 2 per cent. and above followed suit. There was no discoloration. The leaves wilted and died, green. There was no outstanding difference in time between the 2 and the 5 per cent. plants nor between *M* and *S* plants. Five of the eight *M* trees showed injury at thirty-seven days, four of these dying after forty days. There were also five *S* trees injured thirty-seven days after treatment, taking forty-three to forty-five days to die. All plants in soil with 2 per cent. of oil or more were dead in forty-five days.

When sections of all representatives of the series were examined, they were uniform in giving a positive stain for oil only in the cuticle. This fact remained a puzzle until the root systems were exposed in discarding the plants. One per cent. of oil seemed below the critical amount, and the plants which grew in soil with this dosage were alive at the end of the experimental period, and had almost as good root development as the check. But somewhere between 1 and 2 per cent. the lethal concentration is reached. From 2 per cent. upward the tops were somewhat stunted, and the roots had practically disappeared. Only a few limp, brown, straggling ends of the branches were visible. The control plants showed that their roots had elongated and had branched freely during the time of the experiment. In contrast with these the plants in soil with 2 per cent. or more of oil showed no root growth, but a depletion and decay of the original root system. The direct damage by the oil seemed, in the peaches, to be confined entirely to the underground parts but with equally fatal effects.

Discussion

The species examined exhibited marked variation in their reaction to the oil treatment. Where oil actually entered the tissues a slow death followed. This was probably a toxic action since no sections seemed to show oil in enough cells seriously to impede ordinary translocation. Where oil had apparently not gained entrance to the plant, as in the peaches, some other factor must have been responsible. Either there was local toxic action at the roots, or the spread of oil about them was sufficiently complete to prevent translocation of water in the soil and its absorption by the roots, and the plant literally died of drought.

It is not known what relation the oil bears to the soil. A small quantity is evidently held among the soil particles by capillarity, since it is only where larger amounts are added that the oil seemed to move through the soil. It has been shown that as roots take in water from soil adjacent to

them, free water will move in from more remote areas to replenish the supply. This would continue if necessary until only the hygroscopic water remained. Where 3 per cent. or more of oil was added to a pot it was found concentrated about the roots, which would indicate that a similar situation obtained with it.

If some definite part is held by the soil particles it would explain the failure of the lowest quantity to produce results. In the case of tomatoes and peaches the soil seemed able to take up about 1 per cent. of oil by volume without injuring them; but when the amount was greater, the effect was detrimental to the plants.

This investigation opens up many questions particularly of physiological interest which must be left for future studies. Probably abnormalities in temperature, humidity, water content, and nutritive relations would have special meaning when plants are subjected to a test of this sort. It will be valuable to correlate results of outside experiments with the greenhouse data.

Summary

1. Commercial fuel oil has a harmful effect on plants when brought into contact with their roots, through the soil.

2. The effect becomes fatal when the quantity is raised above the critical point for a species.

3. The effect may or may not be due to penetration.

4. If the oil has penetrated the tissues, it is most commonly found in the primary xylem; to a lesser extent it enters the secondary xylem, and the intercellular spaces of the pith and cortex.

5. Contact does not seem to be a primary cause of death.

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BRIEF PAPERS

EFFECT OF TITANOUS CHLORIDE ON THE FORMATION OF CHLOROPHYLL IN *ZEA MAYS*[1]

(WITH ONE FIGURE)

Titanium is found as a constituent of many ores, the chief one being ilmenite and rutile. As the oxide it is generally distributed in surface soils. Clay soils often contain from 0.3 to 0.6 per cent. TiO_2. Titanium stands in tenth place in the series of elements which make up the earth's crust. Many plant organs have been found to contain TiO_2. Potatoes may sometimes contain as much as 0.336 per cent. of their dry weight.

NEMEC and KAS (**2**) in 1923 found that increased crop yields could be obtained by using titanium in the fertilizer either in the form of titanium sodium citrate or the insoluble sodium titanate. They found that the amount of phosphorus, silicon, and aluminum increased and decreased with the titanium content of the plants. The iron content, however, decreased with increasing applications of titanium. This led them to suggest that it might be possible that iron could be replaced by titanium in some plant metabolic processes.

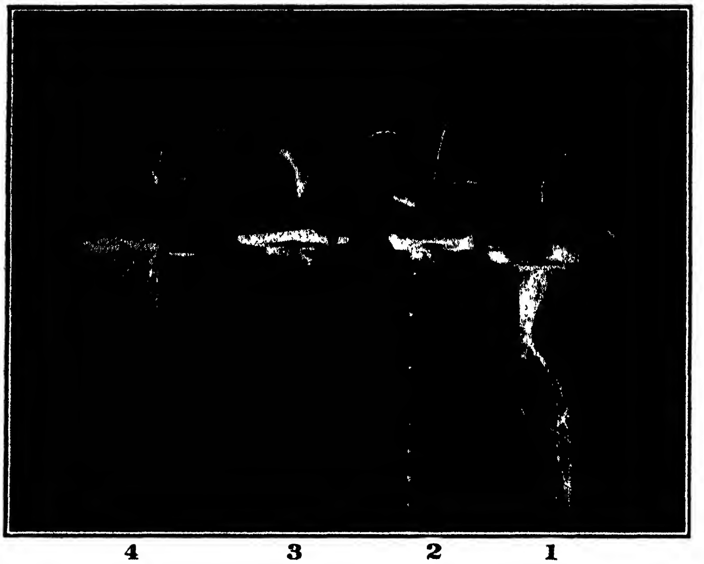
In 1930 SIDERIS (**3**) reported that pineapple seedlings grown in a nutrient solution composed of

0.001 molar K_2HPO_4
0.002 molar KNO_3
0.001 molar $Ca(NO_3)_2$
0.001 molar $MgSO_4$

with five parts per million of titanium but no iron, formed chlorophyll just as well as when iron was added to the nutrient solution. SIDERIS used $TiCl_3$.

About two years ago we set up experiments in our laboratories with the object of testing the effect of titanous chloride ($TiCl_2$) on the formation of chlorophyll in *Zea mays*. We used the same concentration of nutrient solutions as was used by SIDERIS and added 5 and 10 p.p.m. of $TiCl_2$ instead of iron. Our controls consisted of nutrient solution with no titanium and no iron and nutrient solution with iron. This experiment has been repeated five times and 15 and 20 p.p.m. of titanium have been tried. All the results confirm the conclusion that titanous chloride will not substitute for iron in the formation of chlorophyll in *Zea mays*. Furthermore it can be readily seen from figure 1 that the growth of the tops and

[1] Contribution from the Kettering Foundation for the study of chlorophyll and photosynthesis.



4 3 2 1

FIG. 1. Influence of titanous chloride and iron on the growth of *Zea mays.* 1, with trace of iron; 2, without iron; 3, with 5 p.p.m. $TiCl_2$ and no iron; 4, with 10 p.p.m. $TiCl_2$ and no iron.

the root systems is decidedly greater in the nutrient solution to which iron was added.

LEWIS (1) in a discussion of the genesis of the elements points out that nickel was most likely the parent substance for both iron and titanium. On this basis one might be inclined to favor the view that the common origin of iron and titanium might mean that there would be a good chance of substituting titanium for iron in chlorophyll formation in the plant. On the other hand it is most probable that iron acts as a catalyst in the formation of chlorophyll and owing to the fact that there is commonly clear cut specificity in such reactions it is unlikely that there could be a substitution of this type.

For the present this leaves us with no definite evidence that titanium has any value to the plant.—O. L. INMAN, GEORGE BARCLAY, and MALVERN HUBBARD, *Antioch College.*

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NOTES

St. Louis Meeting.—The twelfth annual meeting of the American Society of Plant Physiologists will be held in St. Louis from December 31, 1935 to January 2, 1936. The headquarters of the Society will be at the Statler Hotel. The program has been formulated by the program committee, and includes several sessions for the reading of papers. Joint meetings with the Horticulturists, with Section G, A. A. A. S., and with the Physiological Section of the Botanical Society of America have been scheduled. Appropriate periods for the transaction of necessary business have been provided. The annual dinner of the Society will be held on the evening of December 31. Tickets for the dinner should be obtained promptly on registration in order that accommodations for all may be provided. Concessions on the fares to St. Louis will be published by the A. A. A. S. in Science. Early hotel reservations are advisable because of the large attendance expected for this meeting.

Sixth International Botanical Congress.—The sixth International Botanical Congress which was held at Amsterdam early in September was attended by a large number of botanists from all parts of the world. The official representative of the Society at the Congress, Dr. WALTER F. LOEHWING of the University of Iowa, has consented to prepare an account of the meeting at Amsterdam for PLANT PHYSIOLOGY. If it is possible to do so, we hope to reproduce a photograph of the plant physiologists who were members of the congress.

Regional Section.—A petition for the organization of a new regional section of the American Society of Plant Physiologists will be presented to the executive committee and to the Society for action in the near future. The proposed section would include most of the territory west of the great plains, and Hawaii. Regional organization offers many advantages to the members residing within the region, and stimulates both the scientific and social life of the members. Additional information will be given following the St. Louis meeting.

Life Membership Committee.—The eleventh award of the CHARLES REID BARNES life membership will be made at the St. Louis meeting. The announcement will be made at the annual dinner, as has been the custom in past years. The committee whose duty it is to select the recipient of the award has been appointed by president MURNEEK, and is made up of the following members: Dr. JOHN W. SHIVE, chairman; Dr. E. C. MILLER, Dr. R. B. HARVEY, Dr. H. A. SPOEHR, and Dr. O. F. CURTIS.

Committee Chairmen.—In order to facilitate communications between the members of the Society and the committees which are maintained to carry on its work, the names of the chairmen of the various committees are presented here:

Executive Committee, Dr. A. E. MURNEEK, University of Misouri.
Editorial Committee, Dr. C. A. SHULL, University of Chicago.
Finance Committee, Dr. C. A. SHULL, University of Chicago.
Committee on Chemical Methods, Dr. W. E. TOTTINGHAM, University of Wisconsin.
Committee on Physical Methods, Dr. E. S. JOHNSTON, Smithsonian Institution, Washington, D. C.
Life Membership Committee, Dr. J. W. SHIVE, New Jersey Agricultural Experiment Station.
Program Committee, Dr. E. S. REYNOLDS, Missouri Botanic Garden.
Memorial Committee, Dr. F. M. ANDREWS, Indiana University.

Errata.—A few errors have been noted in the printing of volume 10 of PLANT PHYSIOLOGY. Those which have been found have been listed at the close of the table of contents. It is no doubt an incomplete list, as it has not been possible to devote time to reading the printed pages for this specific purpose. The authors of all papers are invited to examine their own work, and to report all errors found. It is regrettable that any errors slip by, but it is very difficult to avoid at least a few in each volume.

Praktikum der Zell- und Gewebephysiologie der Pflanze.—A new laboratory manual of plant physiology has been written by Dr. SIEGFRIED STRUGGER, Privatdozent in the University of Greifswald. This manual contains 94 experiments, which are arranged in eleven sections. These cover the main problems of general cellular physiology. The first section deals with the methods of preparation of living plant cells for experiment and observation. Then follow sections on plasmolytic phenomena; "intrability" (penetration, permeation, vital staining); permeability; vacuole contraction; plasmoptyse; secretion of pigments; nucleus; protoplasmic and cell sap viscosity; protoplasmic movements; and a section on regeneration, reproduction, and stimulation of cell division. The plants found useful in such experiments are listed in alphabetic order in an index, and a subject index completes the volume. The manual is illustrated with 103 cuts, and offers many good suggestions for successful experiments in the general physiology of plant cells. The publishers are Gebrüder Borntraeger, Berlin (W 35, Schöneberger Ufer 12a), who quote the price as RM 8.5 for cloth bound copies. If the usual foreign discount is allowed, this work is not unduly expensive.

Laboratory Plant Physiology.—A laboratory manual of plant physiology has been prepared by Dr. B. S. MEYER of Ohio State University and Dr. DONALD B. ANDERSON of the University of North Carolina. Part I is the manual, and part II is a supplementary section on laboratory methods. The manual presents 132 experiments, arranged in 18 sections. There are blank leaves for note-taking. The order of presentation is indicated by the sectional topics: Solutions; colloidal systems; plant cells; diffusion, osmosis, and imbition; permeability; the water relations of plant cells; the loss of water from plants; translocation of water; intake of water; the internal water relations of plants; the synthesis of basic carbohydrates; fat metabolism; the absorption and utilization of mineral salts; protein metabolism; digestion; translocation of solutes; assimilation and accumulation; and respiration. Lists of reference text books, manuals, and topical references for each section are included at the close of part I. The outline is attractive, and will be found quite useful. It is lithoprinted by the Edwards Brothers, Ann Arbor, Michigan, and may be purchased from them at a price of $1.75 per copy.

Problems in Soil Microbiology.—A brief monograph in the Rothamsted series on agricultural science has been written by Dr. D. WARD CUTLER and LETTICE M. CRUMP, both of the General Microbiology Department of the Rothamsted Experimental Station. It presents in substance the Aberystwyth Foundation lectures for the 1934–1935 session. There are seven chapters, 104 pages, 18 figures, and a map of protozoon distribution. The chapter headings are as follows: The suitability of the soil for microorganisms; the bacterial population under field conditions; the relation of bacteria to nitrite, carbon dioxide production by soil; the growth of protozoa in pure culture; the behavior of protozoa in soil; and the interactions between soil organisms. This is a very attractive short treatise on soil microbiology, and is an illuminating addition to the available soil monographs. The publishers are Longmans Green and Co., from whom it may be obtained at $3.20 per copy.

The Algae and their Life Relations.—The University of Minnesota Press has issued a volume on the fundamentals of phycology which considers the phylogeny of the algae, the distribution of marine forms in time and space, their classification (based on pigments and food reserves), control of algae in water supplies, and the utilization of algae as food for animals and man. The author of this work is Dr. JOSEPHINE E. TILDEN, professor of botany at the University of Minnesota, who has been a life-long student of the algae. The book is abundantly illustrated with 257 figures. A lengthy bibliography makes it extremely useful for those who desire to

consult the original sources of information. With the subject index it contains 550 pages, and is priced at $5.00 per copy. Dr. TILDEN has dedicated the book to her students, all of whom will appreciate this mark of her affection for them. The book will find many friends.

American Ferns.—An attractive and beautifully illustrated handbook for lovers and growers of ferns has been written by Dr. EDITH A. ROBERTS, Professor of Botany at Vassar College, and JULIA R. LAWRENCE. It presents in popular form just the information that fern growers need for proper management of their ferns. There is a brief introductory chapter on American ferns, and then chapters on how to know, grow, and use ferns in various types of decorative planting. Following these chapters, one finds a key for identification of ferns, a table indicating the time of spore collection and duration of greenness of the fronds, a time table for spore germination, thallus, formation, and appearance of the sporophyte, and a table showing the associational distribution of the species. A helpful glossary of terms is included. This little book will be popular with lovers of our fern flora who desire to know more about them, and to enjoy personal experiences in growing them. The publishers, Macmillan Co., quote it at $2.50 per copy.

Pansoma.—It is inevitable, perhaps, that biological philosophy and speculation is to undergo modification because of our changing ideas as to the ultimate nature of matter and energy. An attempt at the unification of our philosophical ideas has been made by Dr. A. C. LÉEMANN, of the University of Geneva, whose volume of speculation bears the challenging title: Pansoma et la géométrie de l'énergie. The pansome is defined as a three-dimensional energetic entity, indestructible and indivisible, bounded by pansomic surfaces, containing an absolute and constant energy, the only fundamental variability which it is capable of manifesting being that of changing its volume. Dr. LÉEMANN then applies the idea of the pansome to explanations of the physical structure of atoms, the phenomena of heat, ether, relativity, inertia, electricity, magnetism, and radiation.

He then passes on to apply the new philosophy to cosmology, causality, affinity and valence, crystallization, adsorption, the colloidal state, catalysis, vital phenomena, karyokinesis, organogenesis, genetics, mutations, and evolution. In the later sections he deals with nervous and muscular phenomena, paratonic reactions (tactisms and tropisms, etc.), and closes with an application of pansoma to psychology.

Here is an unlimited opportunity to enjoy a new philosophical thrill. The book is written in clear style, and it is broken into many brief sections which will facilitate reading and meditation. It comes in paper binding, and is issued by Libraire Georg & Co., Geneva, at a price of 15 Swiss francs.

Nations Can Live at Home.—Under this title Dr. O. W. WILLCOX in a small volume provides a solution for the problem of war. Incidentally the book emphasizes again his ideas as to how the agricultural lands of the earth should be utilized. There are eight chapters: The Malthusian bogey is extinct!; unfortunately, the Malthusian bogey is not yet extinct; when agronomic science is bankrupt; what the agrobiologist really has; delimiting the population problem; expanding the primary limit; peoples beyond the threshold (Great Britain, Italy, Germany, Japan); and the price of peace. An appendix presents crop yield as a phase of the mass action law. WILLCOX says wars are fought for foods and raw materials. Nations lack foods because of incompetent agriculture. To overcome this incompetence WILLCOX advocates the "divulgation of agrobiologic information." He advocates this especially for the "sore spots" of the earth where production is most ineffective and population pressure most grievous. By bringing in agrobiologic effectiveness, nations can live at home, and not need to war for their supplies.

It must not be forgotten, however, that nations that decide to be self-sufficient are bound to travel a hard, self-sacrificing road, which usually means a hard life for people generally, and a low standard of living. Nations *can* sometimes live at home, but it isn't a desirable life if it means that the nation is to depend entirely upon its own resources for everything. Even without population pressure we would still probably have wars. Incidentally, some of the assumptions made in WILLCOX's version of agrobiology do not stand close scrutiny. As a cure-all for the economic ills of mankind it would probably fail.

This volume is printed by the W. W. Norton Co., New York, and is one of a series of volumes edited by ALVIN JOHNSON. The price is $2.75 per copy.

GENERAL INDEX[1]

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[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The H. W. Wilson Co., New York.

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